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Cover photo: Female shamrock orbweaver, Araneus trifolium (Araneidae), from northeast United States, wrapping an ant that had been on its nuptial flight. Photo by Joe Warfel.

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REVIEW

Spider silk: a brief review and prospectus on research linking biomechanics and ecology in draglines and orb webs

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Abstract. Spiders construct a wide variety of silk structures, ranging from draglines to prey capture webs. Spider silks rank among the toughest materials known to science, and these material properties are critical for understanding how silk structures, such as webs, function. However, the mechanics of spider silk are often ignored in the study of webs. This review aims to show how the material properties of silk proteins, the structural properties of silk threads, and the architectures of webs ultimately interact to determine the function of orb webs during prey capture. I first provide a brief introduction into spider silk and how to characterize its material and structural properties. I then examine the function of draglines as "lifelines" to provide a well-understood example of the interaction of material and structural properties in silk function. Next, I examine how orb webs function in prey capture by first intercepting insects, then stopping their kinetic energy of flight, and finally retaining the insects long enough to be subdued by spiders. I show how variation in the material and structural properties of silk acts synergistically to facilitate the stopping and retention potentials of orb webs, and why this can occur in opposition to how orb webs intercept prey. Finally, I summarize why information on the material properties and structures of silk threads needs to be better incorporated into future investigations of spider webs in general.

Keywords: Biomechanics, flagelliform, major ampullate, orb web, prey capture, protein, spider silk

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1. INTRODUCTION

Silks are critical for the survival and success of the world's more than 41,000 species of spiders (Platnick 2011). The purpose of this review is first to provide a brief introduction to silk to serve as a primer for biologists studying spiders in the field, and then to examine some of the critical questions about spider ecology and evolution that can only be addressed by incorporating an improved understanding of silk production and mechanics. I first explore how silk mechanics relates to the relatively simple function of dragline "lifelines" as a wellunderstood example. I then focus specifically on the modern orb web and the silks used to produce it because orb-weaving spiders are the model system for studies on spider silk, and the functions of orb webs are much better investigated by biologists in the field compared to any other web type. I also focus primarily on the most recent research because historical perspectives are already available for spiders' silks (Gosline et al. 1986; Craig 1997; Hayashi et al. 1999; Vollrath 1999; Hu et al. 2006; Vollrath & Porter 2006; Eisoldt et al. 2011), web ecology and evolution (Shear 1986; Eberhard 1990; Wise 1993), and, more recently, the interface between webs and silk (Craig 2003; Vollrath & Selden 2007; Brunetta and Craig 2010; Blackledge et al. 2011; Harmer et al. 2011).

All spiders produce silk throughout their lives, and most are capable of spinning multiple types of silk threads. Spider silk threads are extruded from discrete glands through individual spigots located on their abdominal spinnerets. The silk threads are assembled nearly instantaneously from liquid feedstocks, or "dopes", of protein at ambient temperatures and without caustic chemicals (Eisoldt et al. 2011). Spider silks rank among the toughest energy absorbing materials known (Vollrath & Porter 2009), requiring up to 7–10 times more energy to fracture than an equivalent volume of synthetic Kevlar (Agnarsson et al. 2010). The substantial interest in spider silk is therefore primarily motivated by the potential to exploit spider silks' incredible mechanical properties for applications

ranging from high performance textiles to medical devices (Altman et al. 2003; Kluge et al. 2008). As a result, we now have a fairly robust set of hypotheses to explain the process of fiber assembly and the molecular basis behind the high performance for at least one type of silk – the major ampullate dragline silk used by orb-weaving spiders as the frameworks of their webs (Vollrath & Porter 2009; Eisoldt et al. 2011). Understanding of the silk gene family that encodes most spider silk proteins, commonly termed "spidroins" because they are spider-specific and fibrous, has expanded substantively in recent years (Gatesy et al. 2001; Ayoub et al. 2007; Garb et al. 2010). Yet, research linking silk to the function of structures built by spiders – particularly prey capture webs, draglines, and egg sacs is generally lacking (e.g., Harmer et al. 2011).

2. SPIDER SILK STRUCTURE AND PRODUCTION

Silk production is broadly distributed among arthropods, evolving independently in several orders of insects, crustaceans and araehnids (Craig 1997). Yet, silk is only loosely defined as semi-crystalline fibrous proteins that are extruded external to an organism's body. However, the mechanical and biochemical diversity of silks is staggering. Spiders are unique in their reliance on silk throughout their lives, their diverse uses of silk, and their production of toolkits of as many as seven or eight different types of silks, each of which has a unique chemical composition and comes from its own discrete gland(s) and associated spigot(s) (Guerette et al. 1996; Blackledge & Hayashi 2006a; Vollrath & Porter 2006; Dicko et al. 2008). Most spider silk proteins are encoded by members of the spidroin gene family, whose evolutionary history is characterized by bouts of gene duplication followed by strong diversification (Gatesy et al. 2001; Gaines & Marcotte 2008; Garb et al. 2010). However, some recently discovered silk proteins are difficult to homologize to the known spider silk gene family – in particular some of the proteins found in the piriform attachments that cement threads together (Hu et al. 2007).

Various spider silks can match the tensile strength of steel (major ampullate silk), absorb more kinetic energy before rupturing than Kevlar (many types of silks), or reversibly stretch almost as far as rubber (flagelliform silk: Blackledge & Hayashi 2006a). These remarkable properties are explained by both the amino acid sequences of spider silk proteins and the way that those proteins are assembled into fibers. Silk is spun from liquid dope through spigots on the spinnerets of spiders (Fig. 1A). The dope assembles into a solid fiber through a phase shift in the structural arrangement of the spidroins, which interlocks the individual molecules, rather than simply "drying out". Thus, the conditions under which the liquid dope is spun can dramatically influence the molecular structure, and hence performance, of the resulting fibers, even for the same starting dope. While the relative importance of protein composition vs. spinning effects for spider silk properties is sometimes debated in specific contexts, there is a general consensus that both matter. Importantly, this means that plasticity in silk properties could evolve through either mechanism (Tso et al. 2007; Boutry & Blackledge 2008; Boutry & Blackledge 2009).

2.1. Protein composition.—Orb spiders famously produce seven different types of silk fibers and glues that are

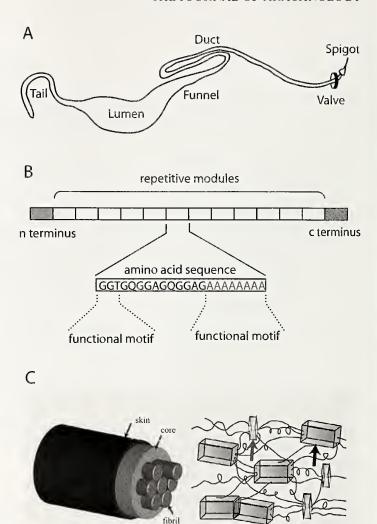


Figure 1.—Production and structure of major ampullate silk. (A) Silk proteins are initially secreted in the tail of the major ampullate silk gland and stored as a liquid dope in the lumen. Shear forces, water uptake, and ion exchange in the funnel and duct cause a phase shift so that new secondary structures form in the spidroins. These structures interlink individual molecules, causing the silk to solidify. A muscled valve provides a final draw down as the fiber exits the spigot, influencing the alignment of the molecules along the axis. From Blackledge et al. 2011. (B) Silk proteins largely consist of repeated sequences of amino acids. Motifs are short sequences of amino acids that are hypothesized to form specific secondary structures in silk such as β -sheets or β -spirals. Several of these motifs are arrayed sequentially to form a repetitive module. Several repetitive modules are themselves arrayed sequentially to form the bulk of the spidroin. This repetitive region is flanked on both ends by ~200 amino acid long terminal regions. From Blackledge et al. 2011. (C) Basic structure of major ampullate silk. A single thread consists of a thin skin of lipids and glycoproteins that surround a core that might show hierarchical layers of organization. Here, the core consists of multiple fibrils. The fibrils consist of a mix of highly crystalline domains embedded in an amorphous matrix. Two levels of crystalline domains are shown here. From Eisoldt et al. 2011.

distinguished by the spigots from which they emerge (Coddington 1989), their mechanical properties (Blackledge & Hayashi 2006a), and their amino acid sequences (Guerette et al. 1996). The general structure of most spidroins consists primarily of a central region of repetitive modules (also called ensemble repeats), with 10–100 of these modules making up

the core region, and flanking N (amino) and C (carboxyl)termini that are ~100-200 amino acids in length (Fig. 1B; Ayoub et al. 2007). The N and C termini are strongly conserved across different types of silks, both within and among species (Gatesy et al. 2001; Garb et al. 2010; Hagn et al. 2010). In contrast, the repetitive modules are often incredibly homogeneous within a particular protein, but highly divergent among silk types (Gatesy et al. 2001). The repetitive modules range from ~50-200 amino acids in length, and short runs of specific amino acids are hypothesized to fold into various secondary structures that influence the performance of the resulting fiber (Guerette et al. 1996; Hayashi et al. 1999).

The semi-crystalline nature of spider silk threads means that much of the repetitive regions of the spidroins are confined in highly organized secondary structures (Fig. 1C). For instance, in the major ampullate silk that comprises draglines and the dry silk frames of orb webs, long repeats of alanine or glycinealanine fold into β-sheets that are hypothesized to stack together and form nanocrystals that interlock individual molecules (Termonia 1994; Grubb & Jelinski 1997; van Beek et al. 2002; Jenkins et al. 2010). The remarkable strength of these crystals is derived in part from hydrophobic interactions that confine hydrogen bonds within the crystal lattice. This confinement is hypothesized to be a key element in explaining how relatively weak hydrogen bonds make for strong silk (Keten et al. 2010). Glycine-rich "amorphous regions" of the spidroins interconnect the crystal forming domains, and here the individual molecules are less confined spatially, often forming loose helices (Simmons et al. 1996; Lefevre et al. 2007). Hydrogen bonding and physical entanglement provide strength and rigidity to the amorphous region, but are easily disrupted as silk is stretched. The end result, at the macroscale, is a fiber that is both strong and stretchy. In stretchier silks, like the flagelliform silk that forms the inner axial fiber of the capture silk in orb webs, the crystal-forming domains are replaced by sequences of amino acids that form β-spirals when proline kinks the amino acid chains (Becker et al. 2003). This greatly increases the overall mobility of the molecules and plays a significant role in the function of orb webs (see Section 3.3). General summaries of the various secondary structures occurring in different types of spider silks are readily available (e.g., Hayashi et al. 1999; Blackledge & Hayashi 2006a; Hu et al. 2006).

Silk threads likely include additional levels of structural organization (Fig. 1C; Sponner et al. 2007). For instance, major ampullate threads are surrounded by sheaths of glycoproteins and lipids (Frische et al. 1998; Augsten et al. 2000). Internally, the core of the thread may be arranged into nanofibrils or contain elongate cavities that may distribute energy and help to prevent crack propagation as energy is propagated longitudinally rather than in the plane of the crack (Li et al. 1994; Frische et al. 1998). The sheath is particularly interesting from a functional standpoint because many spiders can use silk in chemotactile communication (Clark & Jackson 1995; Persons et al. 2002; Gaskett 2007) and the lipids in the sheath are a likely source of these compounds (Schulz 2001).

2.2. Spinning effects.—Silk fibers are assembled from liquid dopes through a process that is reasonably well characterized for the major ampullate dragline silk from *Nephila* and *Araneus*. But, almost nothing is known about the production

of other types of silks (both within these two "model genera" and among other species). Good reviews on the topic are available, although some of the details are controversial (e.g., Knight et al. 2000; Vollrath & Knight 2001; Chen et al. 2006; Eisoldt et al. 2011). I briefly review silk processing because of its importance in ultimately determining silk properties.

Liquid silk is stored within the lumen of the gland at high concentration, up to 50% wt/vol (Vollrath & Knight 2001), with fibroins packed together in micelles that isolate the central repetitive modules of the fibroins in the interior (Jin & Kaplan 2003; Hagn et al. 2010). Solidification of the fiber occurs when the structure of these micelles is disrupted such that the termini can dimerize, and the crystal forming motifs in the central repetitive regions of the proteins are no longer isolated so that their hydrophobic nature instead leads to the formation of β-sheets that stack together and interlock individual fibroins (Knight & Vollrath 1999; Askarieh et al. 2010; Hagn et al. 2010). This process is mediated by a combination of water resorption, ion exchange, drop in pH, and shear flow as the dope passes through an elongated "S"shaped duct (Dicko et al. 2004; Lefevre et al. 2008; Askarieh et al. 2010). A final draw-down of now solid, but still wet fiber occurs at the narrow distal end of the duct, which is mediated in part by a muscled valve in orb spiders (Vollrath & Knight 1999; Ortlepp & Gosline 2004; Pérez-Rigueiro et al. 2005).

Spiders can control the amount of force applied to silk during the final drawn-down (Ortlepp & Gosline 2004; Pérez-Rigueiro et al. 2005). This affects the degree to which spidroins are oriented along the axis of the silk thread and therefore ultimately how stiff and extensible silk threads can be. For instance, the material properties of major ampullate silk can vary almost 50% under different spinning conditions, even within individual spiders (Madsen et al. 1999; Pérez-Rigueiro et al. 2005; Boutry et al. 2011). Thus, the physical processing of the silk dope within the spinnerets of spiders plays a critical role in determining the final structure, and hence also the function, of silk threads (Fig. 1A).

2.3. Supercontraction.—The alignment of the amorphous regions of spidroins along the axis of major ampullate silk fibers is maintained by hydrogen bonding. Thus, the molecular orientation is highly responsive to the environment, particularly to humidity (Vollrath & Porter 2006; Holland et al. 2008; Savage & Gosline 2008; Creager et al. 2010). Supercontraction occurs when water infiltrates silk threads and disrupts hydrogen bonding, thereby mobilizing the spidroins and allowing them to move to a more disordered state (Jelinski et al. 1999; Yang et al. 2000; Eles & Michal 2004). The process is driven by increases in entropy, and the rearrangement of silk molecules occurs quite rapidly. Supercontraction can ultimately cause silk to shrink by up to 50% of its length or to generate substantial forces in confined threads (Work 1981; Boutry & Blackledge 2010). Once a thread has shrunk to its maximally contracted state, it can no longer supercontract unless external forces are applied (Blackledge et al. 2009a), although the silk continues to show a cyclic swelling and contraction that has been implicated for biomimetics (Agnarsson et al. 2009).

The functional implications of supercontraction for webs is debated (e.g., Bell et al. 2002 versus Savage et al. 2004), but remains to be tested in whole orb webs, leaving the potential

"adaptive" value of supercontraction controversial. However, supercontraction was recently hypothesized to provide spiders with a mechanism to control the overall alignment of molecules within silk during the spinning process (Guinea et al. 2005; Liu et al. 2005). Under this scenario, any effect of supercontraction on web function would likely be a byproduct of supercontraction's critical role in silk production. Silk threads are still wet during the final draw-down phase at the end of the spinning duct such that they are effectively already supercontracted so that the amorphous fraction of the silk is still relatively mobile. Spiders can therefore control the overall alignment of the amorphous fraction and how the nanocrystals are packed within it by increasing or decreasing the shear forces applied to the fiber as it exits the spinning duct (Pérez-Rigueiro et al. 2005). Variation in the molecular alignment might therefore account for the high degree of plasticity in mechanical properties that can be exhibited by a spider under different spinning conditions.

3. MECHANICAL FUNCTION OF SILK

The mechanical properties of silk were once challenging to measure due to the small diameters of silk threads, but technology has advanced such that the properties of silk threads as thin as ~300-500 nm are now commonly characterized for studies ranging in focus from phylogenetic variation to phenotypic plasticity to biomimetics. However, data on silk mechanics are still typically lacking from studies focusing on spider web ecology per se. The goal here is to summarize the essentials of the mechanical analysis of silk and some of the implications of variation in the material and structural properties for two common silk devices – draglines and orb webs (Fig. 2).

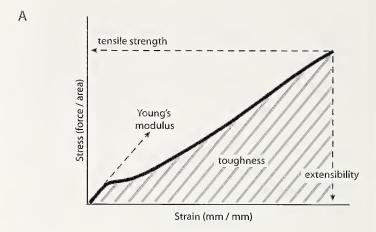
Spider silks are viscoelastic polymers that change their material properties as they are stretched. Therefore, variation in how silks perform when they are initially strained, even small amounts, can be as interesting and important as their behaviors at failure. Most mechanical analyses of silk focus on the stress-strain behavior of fibers because these values are normalized to the dimensions of the sample being tested, which facilitates comparison across different lengths or thicknesses of materials (Fig. 2A). These "material properties" then interact with the "structural properties" (e.g., thickness, length, number of fibers, etc.) to determine the functional properties of devices made from silk, such as how much force a web can sustain or how far it will stretch (Fig. 3; see below).

Stress measures the force generated within a fiber divided by cross-sectional area, while strain measures the ratio of the current to original length of a fiber. Two different methods of calculating stress and strain are common in the silk literature. "Engineering" values are normalized to the original specimen before it was stretched such that engineering stress (σ) is calculated as:

$$\sigma = \frac{F}{A}$$

where F is the applied force and A is the cross-sectional area. Engineering strain (ε) is calculated as:

$$\varepsilon = \frac{\Delta_l}{l_o}$$



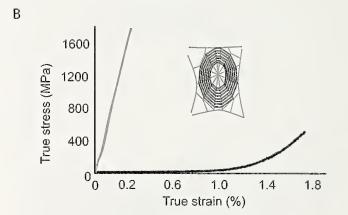
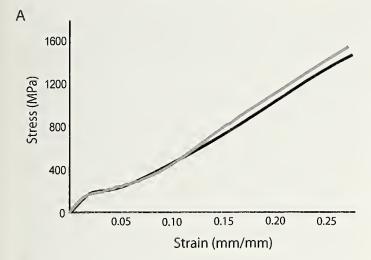




Figure 2.—Material properties of spider silk. (A) Stress-strain test of silk showing four of the most commonly measured material properties. See text for explanation. From Blackledge et al. 2011. (B) Comparison of the material properties of the two fibrous silk constituents of orb webs. The dry major ampullate silk framework (gray) has high tensile strength and stiffness. The wet flagelliform silk core of the capture spiral (black) is orders of magnitude more compliant and extensible. Both silks achieve relatively similar toughness. From Blackledge and Hayashi 2006b. (C) Hysteresis testing of silk. Silk is initially stretched (solid line) and then allowed to relax (dotted line). Energy damping is the proportion of the work performed to stretch a thread (total gray area) to that lost as heat (darker gray). If energy damping was 0%, then the material was perfectly elastic and the dotted line would mimic the original stressstrain test. Major ampullate silk typically has energy damping of ~60%. From Kelly et al. 2011.

where Al is extension of the specimen and l_o is the original length. In contrast, "true" values are normalized to the instantaneous dimensions of the specimen. For true stress, the instantaneous cross-sectional area A_l is substituted for A and



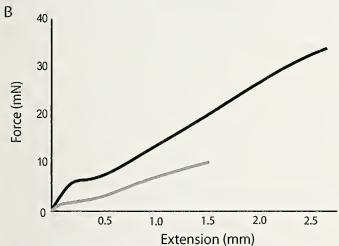


Figure 3.—Material versus mechanical performance of silk. (A) Material properties, such as stress and strain, express performance independent of the dimensionality of a sample to facilitate comparison among samples. Both a thicker, longer sample (black) and a thinner, shorter sample (gray) of the same type of major ampullate silk break at similar stress and strain. (B) However, the actual performance of silk structures also depends on their dimensions, so that it takes substantially more force to break a thicker silk thread (black) compared to the thinner silk (gray). Thus, both the material and structural properties of silk threads need to be considered because natural selection is expected to act upon the performance of structures per se.

is calculated assuming that the thread maintains a constant volume as it stretched. For true strain, the instantaneous length l_i is substituted for l.

These two methods diverge substantially for stretchy materials like spider silk. For instance, a 1-cm-long sample of capture spiral from an orb web could easily stretch 5 additional cm before breaking, which gives an engineering strain of 5 but a true strain of only 1.8 (if that thread broke at engineering stress of 200 MPa then its true breaking stress would be 1200 MPa!). Thus, it is always critical to identify how a given researcher calculates material properties when comparing across studies! Fortunately these measures are easily inter-converted where true stress (σ_t) is calculated as

$$\sigma_t = \sigma(1+\varepsilon)$$

and true strain (ε_t) is calculated as

$$\varepsilon_t = log_e(1+\varepsilon)$$

Much current research reports truestress and truestrain for spider silks. Five aspects of material performance are typically calculated, as summarized in Figure 2A. Tensile strength (also called ultimate strength) and extensibility are simply the stress and strain at which a thread breaks, while the other measures merit further explanation. Young's modulus characterizes the initial stiffness of material, when minor deformations arc highly reversible. Stiffness is calculated simply as the slope of the stress-strain curve. The yield point represents a transition in the behavior of the viscoelastic silk when the molecules begin to flow. This point represents a permanent change in the performance of the silk. Finally, the area under the stressstrain curve measures the toughness of the silk (also called work of extension), and it is the total work necessary to stretch a given volume of silk thread to failure. Toughness is where spider silk really excels. For instance, the tensile strength of dragline silk is only about half that of Kevlar but dragline silk's toughness is about five times greater, with Darwin's bark spider producing silk up to an impressive ten times greater (Agnarsson et al. 2010).

Many spider silks exhibit remarkably high hysteresis. Also called energy damping, hysteresis measures the capacity of a material to transfer kinetic energy to heat as it is deformed rather than storing that energy internally. Hysteresis is calculated simply as the difference in the loading versus unloading energy of a material (Fig. 2C). Major ampullate silk converts about 60% of loading energy to heat as it is stretched, and this amount is relatively conserved phylogenetically, at least among orb spiders where it has been investigated (Kelly et al. 2011). High hysteresis is critical for materials that must withstand high-energy impacts without storing that energy and returning it to the system. Flagelliform silk also has notably high hysteresis, but has to be stretched substantially before hysteresis becomes measureable. Thus, even when stretched to 20%, flagelliform silk acts more like a rubber band, rather than deforming plastically like the permanent thinning that occurs when pulling on a metal wire.

The above values are often called "material properties", but the performance of any silk thread also depends upon its "structural properties", such as thickness and length. By analogy, if you need a stronger rope to support a certain large weight, then you have two choices - you can trade your weaker cotton rope for a material like nylon, which has a higher tensile strength, or you can just get a thicker cotton rope; this is why both material and structural properties need to be considered to understand how webs function. It also leads to the really interesting evolutionary question: how do spiders meet the challenges of web performance in terms of stopping and retaining prey? Are there tradeoffs between structural and material properties? Or, do both evolve in a concerted fashion? These questions are particularly important because the mechanisms by which a spider could alter a web's performance through structural changes in silk lines are often much more apparent than those that could alter material properties. For instance, several species of orb spiders maintain a relatively constant safety factor for their draglines that drops only slightly over their lifetime not by improving

the tensile properties of silk as they grow in size, but simply by spinning thicker threads (Osaki 1996; Osaki 1999; Ortlepp & Gosline 2008). And, cobweb spiders adjust thread diameter when fed "high" versus "low" energy prey (Boutry & Blackledge 2008). On the other hand, several studies indicate that diet can influence the chemical composition of major ampullate silk (Craig et al. 2000; Tso et al. 2005; Guehrs et al. 2008) or aggregate glues (Higgins & Rankin 1999; Townley et al. 2006), although links to variation in mechanical performance of silk are rarely made (but see Tso et al. 2007).

4. SILK "LIFELINES"

Silk plays a fundamental role in how spiders move through the environment – from draglines to bridge lines to ballooning threads. The material and structural properties of silks are critical for each of these functions, and investigations are beginning to unravel how silk functions in both ballooning (Bell et al. 2005; Reynolds et al. 2006) and bridging (Rodríguez-Girones et al. 2010). Draglines present one of the most easily understood structure-function relationships in silk when they act as lifelines for falling or abseiling spiders. Thus, I use draglines to illustrate some of the key concepts to focus upon when considering how silk functions in more complex silk structures such as prey capture webs.

Spiders increase in mass by several orders of magnitude as they mature, and the forces a dragline sustains when stopping a falling spider scale similarly. A safety coefficient describes the degree to which the performance of structures can exceed their functional criteria – for instance, the amount of stress required to fracture a dragline relative to the stress generated by stopping a falling spider (Osaki 1999; Ortlepp & Gosline 2008). Nephila maintain a relatively static safety coefficient that decreases from about 3 to 2 as they mature simply by spinning thicker threads (Osaki 2003). In contrast, the safety factor for Araneus diadematus draglines decreases through ontogeny to the point where they cannot sustain a falling spider (Ortlepp & Gosline 2008). These spiders survive falls by actively releasing extra silk so that they decelerate gradually and some of the work necessary to stop their fall is performed by the muscled valve in the spinning duct itself (Ortlepp & Gosline 2008). Interestingly, major ampullate silk also has a shape memory – high energy damping under torsional (twisting) loading reduces the tendency of silk threads to spin as spiders hang from their draglines when falling or abseiling (Emile et al. 2006; Emile et al. 2007).

Some orb spiders also vary the mechanical performance of draglines based upon what might be an assessment of the risk of falling. Heavier *Argiope trifasciata* spin proportionally thicker draglines when climbing up surfaces compared to smaller individuals, such that spider mass is just less than the force necessary to cause a silk fiber to yield (Garrido et al. 2002). This means that a spider simply hanging from a silk dragline does not cause it to yield, which would cause permanent plastic deformation of the silk molecules. Thus, the performance of the dragline is preserved until a potentially catastrophic fall. *Argiope trifasciata* also spins silk with more consistent material properties when climbing vertically compared to dragline produced when crawling, which should increase the dependability of the total load a dragline could support when a spider falls (Garrido et al. 2002). Thus, spiders

can alter the performance of silk in anticipation of different functions, as also suggested by variation in the mechanical performance of major ampullate silk spun in different regions of cobwebs by *Parasteatoda tepidariorum* (Boutry & Blackledge 2009).

Despite the relatively simple function of silk lifelines, many questions remain. Any lifeline is only as strong as its attachment to the substrate, and draglines are secured via attachment disks produced from piriform silk glands (Coddington 1989). The morphology and chemical composition of attachment disks is beginning to be characterized, but almost nothing is known about their functional properties. One notable exception is the specialized attachment of the capture spiral to radii in orb webs, which can break thereby allowing the capture spiral to slide through them rather than breaking (Eberhard 1976). The piriform attachment disks for draglines are a mix of fibrous and gluey silks and contain spidroins that are unique to the piriform secretions (Blasingame et al. 2009; Perry et al. 2010). How attachment disks actually adhere to the substrate is still unknown.

5. ORB WEBS

The orb architecture is iconic among webs and evolved once in the ancestor of orbicularian spiders (Coddington 1982; Griswold et al. 1998; Blackledge et al. 2009b; Dimitrov et al. 2011). Orb webs played a critical role in the evolutionary diversification of spiders for at least two reasons. First, the development of the discrete aerial framework of major ampullate threads that support orb webs - the radii and frame threads - freed spiders from the constraints of terrestrial sheet webs, thereby acting as a "gateway" for the evolution of novel web architectures (Blackledge et al. 2009b). Equally important, though, is the implication of the mechanical function of the orb web in capturing flying insect prey for the evolution of silks themselves. Most prey-capture webs primarily extend the spider's sensory environment and physically entangle arthropods, slowing the prey enough to facilitate capture by the web owner (Shear 1986). The targeting of flying insects by orb webs introduced two relatively novel selective pressures on silk: 1) dissipation of the massive kinetic energy imparted to orb webs when insects fly into them, and 2) the necessity for strong adhesion to prevent insects from falling out of orb webs.

5.1 Major ampullate silk.—Both the outer framework and supporting radii of orb webs are comprised primarily of silk from the major ampullate gland. Major ampullate silk evolved long before the orb web, ~375 mya (Ayoub & Hayashi 2009 in Garb et al. 2010), and is notably strong and tough even among basal lineages of spiders (Swanson et al. 2006). However, phylogenetic comparison shows that orb spiders' major ampullate silk is significantly stronger and stiffer than other taxa, and this is hypothesized to reflect selection for the silk's energy absorbing function in orb webs (Swanson et al. 2006). These changes in material properties correlate with the origin of a new protein, MaSp2, within orb spiders (Hinman & Lewis 1992; Gatesy et al. 2001). In contrast to MaSpl, whose repetitive elements are dominated by polyalanine and glycinealanine motifs that fold the fibroins into β-sheets, MaSp2 contains a novel glycine-proline-glycine-glycine motif (Gatesy et al. 2001). The presence of the proline typically forces proteins into helical shapes that disrupt the formation of β-sheets, and the tandemly arrayed motifs are hypothesized to fold into molecular "nanosprings" (Becker et al. 2003). This provides greater mobility within the amorphous region of the silk, thereby increasing toughness. The ratio of MaSp1 to MaSp2 expression correlates with at least some of the variation in mechanical performance of major ampullate silk among orb spiders (Liu et al. 2008; Elices et al. 2009).

5.2 Viscid adhesive silk.—The improved performance of dragline silk containing a blend of MaSpland MaSp2 spidroins correlates with the origin of orb webs per se at the base of the Orbiculariae. However, early orb weavers utilized a dry cribellate silk in capture threads that is still produced by Deinopoidea. Bouts of speciation instead correlate far more closely with the evolution of a new adhesive system in the viscid orb web, at the base of Araneoidea (Bond & Opell 1998). The evolution of viscid capture silk occurred early in the history of the orb web, and it is now utilized by 95% of all orb-weaving spiders (Bond & Opell 1998; Blackledge et al. 2009b). Viscid capture silk provides a major increase in the stickiness per volume of capture threads, which likely facilitates prey capture (Opell 1997). This in turn leads to higher growth rates and fecundity in araneoid vs. deinopoid orb weavers (Opell 1997).

Viscid silk achieves stickiness in a fundamentally different manner than cribellate silk. The basic differences are well documented in that cribellate capture threads rely upon physical entanglement and van der Waals interactions, while viscid glue is chemically adhesive (see review in Sahni et al. 2011a). However, a fundamental shift in the mechanics of both the axial threads and their adhesive silk also plays a critical role in adhesive performance. Cribellate capture threads are significantly stiffer and dissipate prey energy primarily through physical breaking of individual fibrils (Blackledge & Hayashi 2006b). Like most adhesive surfaces, cribellate silk resists detaching primarily along the edge of contact with a smooth substrate such as the wing of an insect. Thus, adhesive forces are determined not by the total area of contact but rather by the surface energy along the edge at which detachment occurs and the total number of cribellate fibrils (Opell 1994; Opell & Hendricks 2007).

Viscid capture threads overcome this eonstraint on adhesion through a highly effective suspension bridge mechanism that is enabled by the high elasticity of both the flagelliform axial fibers and the viscous glue droplets themselves (Opell & Hendricks 2007; Opell et al. 2008; Sahni et al. 2010). The viscid glue droplets have their own hierarchical structuring and consist of a core of cross-linked fibrous glycoproteins embedded in a liquid matrix (Opell & Hendricks 2010). Adhesion occurs mostly due to the interface of these glycoproteins with the surface (Vollrath & Tillinghast 1991). As a viscid thread begins to pull away from a surface, individual glue droplets extend greatly before they detach (Sahni et al. 2010; Opell et al. 2011). This allows multiple glue droplets to simultaneously resist pull-off, generating significantly more adhesion (Opell & Hendricks 2007). Furthermore, up to 50% of the total work required to pull a viscid thread free from a surface eomes not from the glue, but instead from the extension of the axial fibers themselves (Sahni et al. 2010). The extensibility of both the glue droplets and the flagelliform silk is enabled by their hydrated states, which are maintained

Table 1.—Theoretical interactions between the material properties of silk proteins, the structures of silk threads, and the architectures of orb webs for each phase of prey capture. The number of plusses or minuses indicates the relative degree to which a particular trait influences a phase of prey capture. Parentheses indicate an influence that is largely indirect and due to the correlation between increased capture area and mesh width/fiber diameter. Note how several traits that positively influence stopping and retention potential have a negative influence on interception.

	Interception	Stopping	Retention
Material properties			
High toughness		+++	+
High extensibility		++	+++
High hysteresis		+++	+
Structural properties			
Thick fibers	_	+++	+
Large droplet size	_		+++
Architectural			
Large capture area	+++	(-)	+/(-)
Narrow mesh width	_	++	++

by a cocktail of hydrophilic salts in the glue droplets (Vollrath et al. 1990; Townley et al. 1991).

The dependence of viscid threads on water for their mechanical function has at least two important consequences. Adhesive forces are highly dependent upon the water content of the glue (Opell et al. 2011) and become optimized at intermediate humidity due to competing processes (Sahni et al. 2011b). Higher water content increases molecular mobility, and hence extensibility of both the axial threads and glue droplets, and facilitates spreading of the glycoproteins, but at the same time also begins to over-lubricate the contact surface. The precise humidity maximizing adhesion is determined at least in part by the salt content of the glues (Sahni et al. 2011b). Thus, variation in salt concentrations per se provides a very simple mechanism by which natural selection could act on silk adhesion, potentially leading to local adaptation to different web microhabitats. Unfortunately, comparative data are mostly lacking, although natural history observations show that the glues of some cyrtarachnine spiders function only at extremely high humidity (Stowe 1986).

5.3 The function of silk in orb webs.—Although orb webs may play roles in courtship, thermoregulation and defense against predators, their primary function is to facilitate capture of flying insect prey. The role of orb webs in prey capture can be understood as a three-step process intercepting, stopping and retaining prey until the insects are subdued by spiders (Blackledge et al. 2011). Success at each stage can be influenced by specific features of orb web architecture and silk mechanics, although general design principles are not always clear and functional tradeoffs are likely (e.g. Blackledge & Zevenbergen 2006; Blackledge & Eliason 2007). General discussion of web architecture and its influence on prey capture can be found elsewhere (Eberhard 1986; Nentwig Heimer 1987; Eberhard 1990; Zschokke 1999; Heiling Herberstein 2000; Blackledge et al. 2011), and Table 1 summarizes some of the significant factors affecting prey capture. In general, design features that facilitate the stopping and retention of prey are largely synergistic or neutral with

respect to one another. However, there are fundamental tradeoffs in how orb web design influences prey stopping and retention versus the initial interception of insects. Generally, spreading silk resources across larger webs with broadly spaced capture spirals should maximize the numbers of insects that fly through a web and contact silk. The most efficient design of an orb web that maximizes the number of prey contacting silk is constructed by spacing threads just larger than the average insect's wingspan (Eberhard 1986). Such a design is in general less visible to insects than more compact architectures, due to the thin diameters of silk threads and droplet sizes (Craig 1986; Craig 1988). However, these features reduce the probability of stopping and retaining prey once the insects are intercepted (Blackledge & Eliason 2007). Larger orb webs also increase the response time of spiders, since they need to navigate greater distances to entangled prey (see Nakata & Zschokke 2010; Zschokke & Nakata 2010 for discussion of spider response times).

A fundamental tradeoff between the interception potential and the stopping/retention potentials of orb webs is evident in comparisons of web architectures and silk mechanics among species. The dominant trend among orb spiders is associated with evolutionary shifts in body size (Sensenig et al. 2010). Larger species produce higher quality silk that is spun into orb webs with high stopping potential. Silk in these webs is packed relatively tightly, and there is a notable correlation in the improvement of the material properties of both major ampullate and flagelliform silk among larger species of spiders. One possible explanation for this pattern is the reliance on relatively large, but rare, insects demonstrated by Venner & Casas (2005) for Zygiella x-notata (Clerk 1757). The reliance on rare, large prey appears reasonably generalizeable for orb spiders - a comparison of diverse spider species ranging more than 20 mm in maximum body length shows that roughly 85% of all biomass captured is composed of only a few insects proportionately similar in size to the spiders capturing them (Blackledge 2011). The kinetic energy of flying insects increases exponentially with their body size as both mass and flight speed increase. The ability of large species of spiders to target large insects depends more on how their web design facilitates the stopping and retention of difficult prey than on the probability of those prey encountering the web (Blackledge 2011). Unfortunately, the smallest orb webs, spun by the Mysmenoidea, are constructed using silk that is too thin to easily characterize using standard materials testing equipment, so nothing is known about the evolution of silk properties and web function in lineages evolving miniaturized body forms.

There is a close correlation between the numbers of rows of capture silk and the numbers of supporting radii in orb webs, with the ratio typically near one. There are two functional explanations proposed for this relationship. One hypothesis is that the constant ratio reflects a continuum between web architectures targeting high energy vs. low energy prey (Craig 1987). Here, species targeting higher energy prey package thicker silk threads more tightly into webs, while webs targeting lower energy prey contain fewer rows of widely spaced capture spiral supported by proportionally fewer radii. However, recent work suggests a compensatory tradeoff where better mechanical performance of silk in more "open"

webs – due both to improved material properties and thicker threads - results in stopping potential per unit area only slightly lower than webs with more tightly packed architectures for species of similar sizes (Sensenig et al. 2010). The second functional explanation does not involve prey capture per se, but instead reflects a constraint imposed by the very high compliance and relatively low tension of the capture spiral. More radii become necessary to hold the capture spiral in place as mesh width narrows to prevent adjacent capture threads from adhering to one another, thereby degrading web function. The absolute distance that a segment of capture spiral sags is proportional to its length (actually length cubed), so that shorter distances between radii in an orb web reduce the probability that capture silk segments can stretch and potentially entangle one another (see Rodríguez-Girones et al. 2010 for a similar discussion of how silk elasticity might constrain bridging thread length).

The retention time of insects in orb webs is typically quite short, often less than one second, providing little time for spiders to sense and subdue prey (Rypstra 1982; Blackledge & Zevenbergen 2006). The role of adhesive silks in prey retention is most investigated at the transition from cribellate to viscid capture silks (Opell 1997; Opell 1998; Opell 1999). Comparative studies have only recently begun within the viscid silk producing Araneoidea (e.g., Opell et al. 2008; Agnarsson & Blackledge 2009; Opell & Hendricks 2009). The total adhesive force generated by viscid capture threads scales remarkably closely with $\sim 80\%$ of breaking force for the underlying axial fibers, suggesting that the glue has evolved to safely detach from prey before the threads break, thereby maintaining the ability of the silk to re-adhere to struggling prey (Agnarsson & Blackledge 2009). Because of the close correlation between the tensile strength of capture spiral and radial silks (Sensenig et al. 2010), orb webs with high stopping potential should in general have higher retention potential, too. Relating interspecific variability in thread stickiness to web function is difficult, however, because retention times vary so much among different taxa of insects, even when the insects are superficially similar in terms of body size or flight speed (Blackledge & Zevenbergen 2006). This variability is caused by differences in the flight and escape behaviors of insects, as well as the details of how cuticular features interact with adhesive silk (Opell & Schwend 2007). In general, variation in the average mesh widths of orb webs typically does not correlate closely with prey size (e.g., Nentwig 1983; Prokop 2006; but see Herberstein & Heiling 1998). However, some generalizations can be made about the effect of capture spiral spacing on prey retention from experiments altering the mesh width in webs by selectively cutting capture spiral rows. Narrow mesh width can increase retention times significantly for certain taxa of insects (Blackledge & Zevenbergen 2006), but it can also have surprisingly little effect on prey capture in the field. Blackledge & Eliason (2007) found that the weight gain of Argiope aurantia foraging in the field on webs with every other row of capture spiral removed did not differ in weight gain over the course of a single day compared to spiders on control webs. However, control spiders were significantly more likely to have larger prey wrapped in the web that they could continue to feed upon (Blackledge & Eliason 2007). This suggests that an important selective factor on the spacing between rows of capture silk is not the ability of orb webs to retain average prey, but rather rare, large prey.

Finally, the mechanical interaction of orb webs with insect prev does not occur in isolation from other factors affecting prey capture. For instance, the microhabitat location of orb webs helps to determine prey availability and can influence the stopping potential of webs when insects "ricochet" among closely spaced webs (Uetz 1989; Rao 2009). The visibility of orb webs influences how effectively insects avoid webs and potentially their impact energy. The degree to which silk is visually attractive or repulsive to insects is remarkably controversial (see reviews in Herberstein et al. 2000; Blackledge et al. 2011). However, many features that improve stopping and retention potentials of webs, such as thicker, more tightly packed silk threads and larger glue droplets, clearly enhance web visibility, thereby potentially reducing the webs' interception of insects (Table 1). Lastly, the attack behaviors of spiders vary greatly among taxa (Barrantes and Eberhard 2007) and are plastic (Robinson & Olazarri 1971), such that response time and running speed should vary with the sizes and retention potentials of orb webs (Zschokke et al. 2006; Nakata and Zschokke 2010). The degree to which web visibility and spider attack behaviors may coevolve with orb web mechanics is largely unknown.

5.4 Beyond orb webs.—The focus here is primarily on the function of silk in spider orb webs, yet orb webs are only a small fraction of all the silk structures produced by spiders, many of which function in unique but relatively unexplored ways. For instance, the sticky gumfooted threads in the cobwebs of theridiid spiders act as spring-loaded traps where energy is stored within the structure of the cobweb during prey capture (Argintean et al. 2006), rather than dissipated as in orb webs. The glue coating the gumfoot threads also differs in microstructure and adhesive response to humidity (Sahni et al. 2011b), as well as containing unique proteins (Hu et al. 2007). Unfortunately, nothing is known about the diversity of mechanical properties among the silk of different species of cobweb spiders, let alone anything about functional differences in their webs. The lack of knowledge is even more apparent when considering other types of prey capture webs, such as the many types of sheet webs produced by spiders, and non-prey capture structures such as egg sacs. Clearly there is a great need to expand research on both silk and webs "beyond the orb" (e.g. Eberhard 1990).

6. SUMMARY

Silk research is driven primarily by its biomimetic potential in industry and medicine (Hinman et al., 2000; Altman et al., 2003; Vollrath & Porter 2009), while research on spider webs is motivated primarily by the need to understand the ecology and evolution of these unique predators (Shear 1986; Wise 1993). Integrating these approaches is both advantageous and necessary (Harmer et al. 2011). Orb webs function in prey capture by first intercepting insects, then stopping their kinetic energy of flight, and finally retaining the insects long enough to be subdued by spiders. Each step in the process is determined by an interaction between the material properties of silk proteins, the structural properties of silk threads, and the architectures of webs. These interactions are largely synergistic for the stopping and retention potentials of webs,

but there is likely substantial conflict with respect to how silk structure and web architecture influence interception. Regardless, information on the material properties and structures of silk threads need to be better incorporated into future investigations of orb webs.

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Parasitoid suppression and life-history modifications in a wolf spider following infection by larvae of an acrocerid fly

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Abstract. Flies of the family Acroceridae are specialized internal parasitoids of spiders. We infected hatchlings of wolf spiders *Pardosa prativaga* (L. Koch 1870) (Araneae: Lycosidae) with larvae of *Acrocera orbiculus* (Fabricius 1787); most hosts were infected by a single larva, but others endured multiple infections of up to eight larvae. The infected spiders and a group of uninfected control spiders were raised in the laboratory for up to 23 weeks. We found that most (81%) spiders infected by only one larva were able to suppress the infection, whereas most multiple infections (73%) were "successful" (i.e., a larva emerged or was recovered by dissection, perhaps from a prematurely dead spider). Infected spiders had their survival reduced in proportion to the infection load, but the reduction was not significant if the infection was suppressed. Infected spiders had higher growth rates than uninfected, and growth stimulation was proportional to the number of initially infecting larvae and independent of whether the larva was suppressed or not. Due to these patterns, we suggest that growth enhancement results from the spider's mobilization of extra resources for combating the infection rather than parasitoid manipulation of spider growth. Spiders with multiple infections took longer to mature than uninfected spiders, and the pattern of instar durations was changed compared with that of control and singly infected spiders. As multiple infections were important for the parasitoid's success, we suggest that the parasitoid fly's habit of laying eggs in large clumps may be an adaptation to increase the chance of success via multiple infections.

Keywords: Acroceridae, life history, Lycosidae

The interplay between a parasitoid and its host is intriguing. On one hand, a parasitoid's survival and growth depend entirely on the successful survival and growth of its host (Godfray 1994; Brodeur & Boivin 2004). On the other hand, a host in excellent condition may more easily avoid a parasitoid infection in the first place, but also be more effective at suppressing a current parasitoid infection. Since a successful parasitoid is deadly, it is always in the host's interest to get rid of it as early as possible. The parasitoid should be virulent enough to avoid suppression from the host, but at the same time minimize the physiological costs to the host and selectively manipulate specific aspects of host physiology and behavior (Godfray 1994; Brodeur & Boivin 2004). Though we expect a parasite infection to be costly to the host, there should be strong selection on the parasitoid to reduce the negative impact of the infection until it finally kills the host (Slansky 1986). If possible, it might even be advantageous for the parasitoid to stimulate the growth of the host. This would either provide more resources for the parasitoid that may obtain a larger body size, or reduce the time of development and thus enhance the chances of survival. Growth enhancement is common for hosts of gregarious parasitoids, whereas growth inhibition and reduced activity is the rule for hosts of solitary parasitoids (Slansky 1986; Harvey et al. 1999; Harvey 2000), reflecting a difference in nutritional demands of multiple versus single parasitoids, though this may also depend on the size of the host (Harvey et al. 2010).

Flies of the family Acroceridae are specialized parasitoids of spiders, mostly cursorial species (Schlinger 1987). Most published accounts of these animals are lists of parasite-host relationships and data on spider infection rates based on rearings of field-collected spiders. Information on the interactions between the spider host and the developing parasitoid is very limited (Schlinger 1987) because the adult flies are difficult to maintain in the laboratory. Chance events have

allowed us to obtain eggs and first-instar larvae (planidia) from a few mated females of the species Acrocera orbiculus (Fabricius 1787) from the field. In the first place this allowed us to observe its unique mode of entrance into the spider host (Nielsen et al. 1999); subsequently, we infected a large number of wolf spider hatchlings and thus were able to get some information on the fate of the parasitoids after infection, as well as on how the life history parameters of the spider are modified by the infection. Since some spiders were singly and others multiply infected, we were also able to analyse how parasitoid success and spider life history depend on the infection load. We were interested in how effective the spider host may be in suppressing parasitoid infections, and whether the parasitoid is able to manipulate the spider's growth pattern for its own benefit. Considerable attention has been devoted to modifications of host behavior (Godfray 1994; Thomas et al. 2005), also in spiders (Eberhard 2000, 2010), but less attention has been paid to how spider life histories are molded by parasitoid infection and the relative role of parasitoid and host strategies in these modifications.

METHODS

The parasitoid.—Acrocera orbiculus has a wide distribution covering both the Palaearctic and the Nearctic (Nartshuk 2010). Adult females lay clumps of several hundred eggs in the vegetation, and the tiny, hatched planidia larvae (0.3–0.4 mm) move about actively searching for a potential spider host. Having found a potential host, the larva first attaches itself by the mouthparts, usually to a leg, to first become an ectoparasite. It then becomes an internal parasite after the first molt when the amoeboid second instar larva enters the spider's haemolymph via the tiny hole where the planidium larva's mouthparts are attached (Nielsen et al. 1999). This second instar larva enters the abdomen (via legs, prosoma and pedicel) and takes up a position near the booklungs. Here it

grows to its final size, mainly during the third and fourth instar. The fully grown larva exits the dying spider to pupate outside the carcass.

Procedure.—Two females of *A. orbiculus* were collected live at Mols, Denmark, and brought to the laboratory. During subsequent days they laid a large number of eggs in the collecting vials. After approximately two weeks at 21°C the eggs hatched into active planidia larvae. Approximately 100 hatchlings of the wolf spider Pardosa prativaga (L. Koch 1870) (Araneae: Lycosidae) were released into the vials and left there overnight to allow the planidia to infect the spiders. The spiderlings were inspected for attached larvae under a binocular microscope and transferred to individual breeding vials with a plaster bottom. The number of attached larvae varied between 0 and 8 per spider; thus the parasitoid load was not strictly controlled. We had 39 uninfected spiders, 37 infected with 1 larva, 7 with 2, 4 with 3, 1 with 4, 2 with 5, and 1 with 8 larvae. The spiderlings were raised in the vials at 25 °C and a 16L:8D photoperiod, being fed fruit flies ad libitum and watered 2–3 times per week. The non-infected spiderlings served as a control group. The fruit flies used were nutrientenriched by being raised in a mixed medium of Carolina Biological Supply *Drosophila* medium (Formula 4–24) and crushed dog food. This mixed medium is nutritionally optimal for survival and growth of the spiders (Mayntz & Toft 2001). The spiders were weighed weekly from the start of the experiment and up to 23 weeks. At this time some spiders had died; acrocerid larvae had emerged from others in order to pupate; the remaining spiders were killed at this time. Spiders that died, as well as those that remained at the end, were preserved in alcohol and subsequently dissected to check for presence and number of acrocerid larvae in the abdomen.

Statistical analyses.—JMP 8 was used for the statistical analysis. We distinguished three groups of spiders according to the initial infection load ("# infecting larvae"): control (Acr = 0, non-infected), single infection (Acr = 1), and multiple infection (Acr = 2+, 2-8 larvae). Spiders were also grouped according to the success of the parasitoid, defined by the result of the dissections: no larva (L = 0) or one or more larvae present (L = 1+). Survivorship was analyzed by a Wilcoxon test. The growth curves were analyzed by repeated measures ANOVA of the weekly weight measurements. The time* parasite load (# initially infecting larvae or # surviving larvae) interaction terms were used to indicate significantly different growth patterns. Due to mortality, meaningful tests could only be made on data up to week 15. Developmental parameters were analysed by ANOVA and t-tests; the data were checked for homogeneity of variances (Levene's test); when this criterion could not be met, the Welch t-test was used.

RESULTS

Parasitoid suppression.—Only three fully developed acrocerid larvae emerged from their spider hosts. However, several spiders that died during the experiment or were killed when the experiment ended turned out by dissection to contain one or two acrocerid larvae of intermediate or large (i.e., close to fully grown) size in their abdomens. These are here considered successful. However, many of the originally infected spiders turned out to have no acrocerid larva inside, suggesting that

they had been able to suppress the parasitoid infection. Most of the spiders originally infected with only one larva (30 out of 37 = 81%) got rid of it, while this was the case for only a minority (4 out of 15 = 27%) of multiply infected spiders ($\chi^2_1 = 13.96$, P < 0.0002). All four spiders originally infected with 4–8 planidia had larvae in their bodies when dissected; of those infected with 2–3 planidia, 4 out of 11 spiders had suppressed the infection. Of the three emerging larvae, two were from singly infected and one from a doubly infected spider.

Spider survival.—Survival of the spiderlings was higher in the control group than among infected spiders (Wilcoxon test, $\chi^2_2 = 23.0$, P < 0.0001; Fig. 1) and directly related to the number of infecting larvae (Acr = 0 vs. Acr = 1: χ^2_1 = 4.44, P = 0.0351; Acr = 1 vs. Acr = 2+: χ^2_1 = 8.08, P = 0.0045). Among the singly infected spiders, survival was much better in those that suppressed the infection than in those that did not $(\chi^2_1 = 7.7, P = 0.0054)$, and the same was true for multiply infected spiders in spite of low sample size $(\chi^2_1 = 4.7, P =$ 0.0296). Survival of singly infected spiders that suppressed the infection was intermediate between control spiders and all singly infected spiders and did not differ from that of control spiders ($\chi^2_1 = 1.0$, P = 0.31). Thus, survival seems to be determined by whether or not the larvae survive and grow in the spider's body. Therefore, overall survival of singly infected spiders was only slightly reduced because most spiders suppressed the infection, whereas multiply infected spiders (with 2–8 larvae initially), of which fewer succeeded in suppressing the infection, had more strongly reduced survival (Fig. 1).

Spider growth.—Spiders infected with acrocerid larvae had higher growth rates than control spiders, and the more larvae initially attached themselves, the higher the growth stimulation (repeated-measures ANOVA, Wilk's $\lambda = 0.3$, P < 0.0001; Fig. 2). Growth stimulation was significant in the spiders that suppressed the infection, both as regards the singly (contrast: F = 2.39, P = 0.0126) and the multiply infected (contrast: F = 4.41, P < 0.0001) spiders. There was no difference in growth stimulation, however, between the spiders that remained infected and those that suppressed the infection (singly infected spiders: F = 1.59, P = 0.18); multiply infected spiders could not be tested due to small sample sizes, but show a trend of reduced growth stimulation in spiders with a growing larva (Fig. 2).

Spider development.—For individuals that completed development to the adult stage, total development time was independent of the number of initially infecting acrocerid larvae (ANOVA, $F_{2,49} = 0.38$; P = 0.69) and of spider sex (Welch t-test, t = 1.85; P = 0.0734). Spiders with a successfully developing acrocerid larva took longer to reach maturity than uninfected spiders (142.0 vs. 122.8 days; t-test, t = 4.82, P = 0.0019, n = 52). The first three instars were rather short (1–2 weeks) in the normal pattern of juvenile development unaffected by parasitoids, and were followed by two very long (5–6 weeks) and an intermediate (ca. 3 weeks) instar. This pattern was repeated in singly infected spiders (most of which suppressed the parasitoid), but not in the multiply infected spiders (Fig. 3). In the latter, the fourth instar was as short as the three previous ones, and the fifth and sixth instars were prolonged. Spiders matured after 5-7 juvenile instars; the maturation instar was independent of

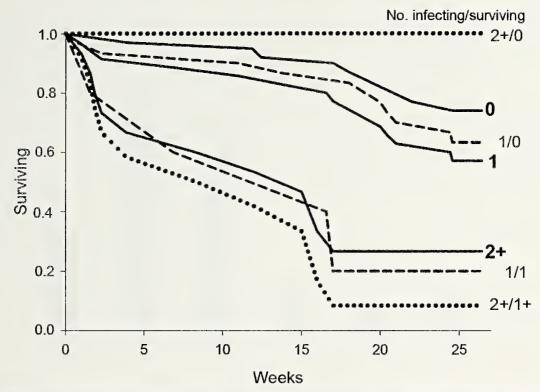


Figure 1.—Survivorship curves for groups of spiders subjected to infection by larvae of the acrocerid fly *Acrocera orbiculus*: uninfected (control) spiders (marked 0); spiders originally infected with one (marked 1), split into those that suppressed the infection (1/0) and those that did not (1/1); spiders originally infected with 2–8 (marked 2+) larvae, split into those that suppressed the infection (2+/0) and those that did not (2+/1+).

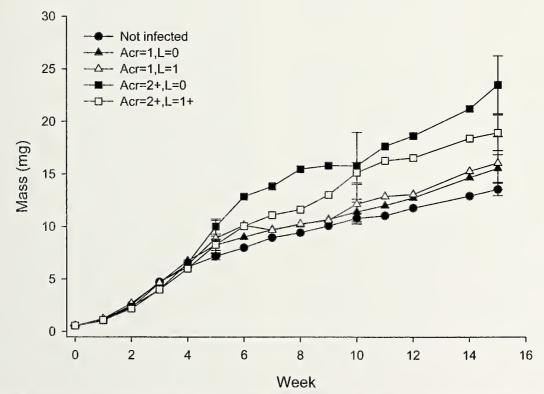


Figure 2.—Growth curves for control spiders and spiders infected with planidia larvae of an acrocerid fly. Acr: number of initially infecting larvae (0, 1 or \geq 2); L: number of growing larvae in the spiders' body (0, 1 or \geq 2). For clarity, error bars (SE) indicated only at weeks 0, 5, 10 and 15.

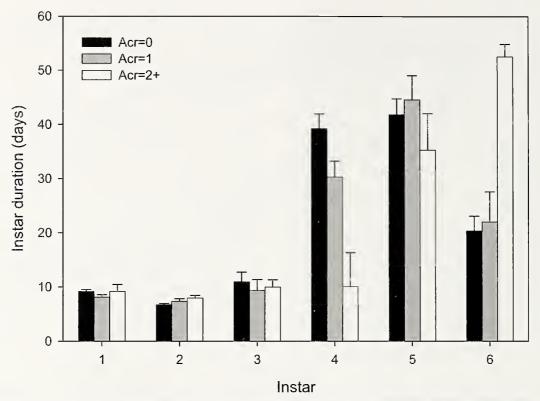


Figure 3.—Duration (mean + SE) of juvenile instars of the wolf spider $Pardosa\ prativaga$ depending on initial infection load by planidia larvae of an acrocerid fly. Acr = 0: uninfected control spiders; Acr = 1: spiders infected with 1 larva; Acr = 2+: spiders infected with 2-8 larvae. Infection happened at the start of instar 1.

sex ($\chi^2_2 = 0.11$, P = 0.95), number of initially infecting larvae ($\chi^2_4 = 5.62$, P = 0.23) and whether there was a surviving larva ($\chi^2_2 = 0.96$, P = 0.62).

DISCUSSION

Most spiders infected with a single parasitoid succeeded in suppressing the infection and in obtaining a close to normal life in terms of survival and growth. It is unknown to what extent the spiders' high success of parasitoid suppression will apply also to natural conditions. The ad libitum feeding of the spiders with nutrient-enriched flies may have strengthened the spiders' immune system (Slansky 1986) to more effectively combat the infection. Several environmental conditions in the laboratory also differed from those in the field, but it cannot be decided whether these have benefitted the spider or the parasitoid. The wolf spider may also be an inferior host for A. orbiculus. Species of Acrocera have a wide host range, including seven spider families (Schlinger 1987), but the relative suitability of these potential hosts is unknown. The greatly increased mortality of successfully infected spiders compared with control spiders and unsuccessfully infected spiders may indicate a possibly low suitability of *P. prativaga*. The fact that we only allowed infection of spider hatchlings may also have been of importance, as the instar infected is known to affect the suitability of insect hosts of parasitoids (Harvey 2000; Harvey et al. 2010; Khafagi & Hegazi 2008). It prevented the acrocerid larva from immediate growth and development and enforced upon it a period of developmental arrest, waiting for the spider to grow sufficiently for the parasitoid to complete its development. We have no information on the optimal host size for infection, or

how A. orbiculus synchronizes its life cycle with that of its spider hosts.

Multiple infections had higher costs for the spiders in terms of reduced survival, but were much more successful from the fly's point of view than single infections. More larvae developed from multiply than from singly infected spiders in spite of many fewer multiply infected spiders. Superparasitism thus seems advantageous, probably because it may help to break the host's resistance, even in a situation where only one parasitoid per host can complete development, as known also from insects (Blumberg & Luck 1990; Khafagi & Hegazi 2008). The adult fly is of approximately the same size as the adult spider, probably eliminating the possibility that more than one fly can successfully emerge. It may therefore be hypothesized that the females' habit of laying eggs in large clumps on the vegetation is an adaptation that increases the chance of multiple host infection. Multiple infections will lead to strong competition between the larvae with only one of them being successful. However, when they occur, multiple infections are likely to be by larvae from the same batch of eggs; i.e., they will be kin (at least half-sibs), and even the larvae that succumb will gain an inclusive fitness benefit. In some cases two acrocerid larvae were found in a spider's abdomen, but in no case were these of the full size ready for pupation, as was common with single larvae. It is therefore doubtful if enduring multiple infections ever lead to pupation. The benefit may be restricted to cases where competition between the larvae results in an early winner.

Contrary to most solitary hymenopterous parasitoids of insects (Slansky 1986; Harvey et al. 1999), acrocerid infection

caused a stimulation of spider growth and thus an increased rate of feeding. Multiply infected spiders also had enhanced growth compared with singly infected spiders; i.e., the stimulation depended on the infection load. Since the body mass measurements included the combined masses of the spider and the parasitoid larva, the enhanced total growth might result from simply adding the growth of the larva to that of the spider. Enhanced growth of infected hosts may be interpreted as a result of parasitoid manipulation through which the parasitoid adjusts the resources available from the host for optimizing its own growth. Thus, Harvey (2000) and Harvey et al. (2010) found that parasitoid infection reduced the growth of large, but stimulated that of small, host species. Stimulation of growth in P. prativaga might thus be due to infection of the hatchling instar. However, the data do not support this view: growth enhancement occurred as a result of the original infection, independently of the fate of the parasitoid; it was seen also in the singly infected spiders that suppressed the parasitoid, and parasitoid larvae successfully developing in the spider's body did not further increase the spider's growth rate. This indicates that growth enhancement might be part of the process by which the spider fights the parasite. Our experimental spider, Pardosa prativaga, is known to be able to quickly catch up with a setback in growth and development following periods of food stress (toxic or nutrient deficient food) by compensatory growth (Jespersen & Toft 2003). We suggest that a similar process of enhanced resource acquisition and mobilization is induced in response to parasitoid infection, perhaps induced already by the planidia larva. The enhanced growth is obvious already from Week 5 (Fig. 2), which is probably much earlier than the period of intense growth of the parasitoid larvae. Thus, it may not so much be a reduction in resources for its own growth that stimulates the spider, but rather the increased nutritional demands of the spider's immune system during encapsulation (i.e., resisting a parasitoid infection) of the parasitoid (Slansky 1986; Strand & Pech 1995). Since there may be a cost of increased growth per se (Higgins & Rankin 2001), the reduced survival of infected spiders may be explained not only by a negative "health effect" due to the infection but also by this growth cost, or both. This line of argument interprets the enhanced growth rate of the spiders as a costly response likely to be paid later in terms of reduced reproductive success (Metcalfe & Monaghan 2001). Encapsulation was also found to have costs in a pyralid moth (Harvey et al. 1996).

The study has revealed an impressive ability of the wolf spider to suppress an infection by the acrocerid fly. This comes on top of previously reported pre-infection attacks on the larvae as prey, and early post-infection ability to mechanically free itself from attached larvae (Nielsen et al. 1999). Physiological and life history costs to spiders that suppressed their infection seemed minimal. Unfortunately, we did not also have the opportunity to test whether parasitoid suppression modified the spider's subsequent reproductive life. At the same time the parasitoid had a heavy impact on the spiders in terms of higher mortality. It remains to be established whether A. orbiculus has more suitable hosts than P. prativaga available in the northern European part of its distribution. If not, this may explain its rarity here.

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Reproductive activities impair immunocompetence in *Physocyclus dugesi* (Araneae: Pholcidae)

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Abstract. When host organisms mount an immune response, they incur energetic costs. Theory predicts that these costs result in trade-offs between investment in life history traits (such as growth and reproduction) and investment in immune response. Recent empirical work investigating whether immune ability is impaired during sexual activity in invertebrates does not uniformly support this prediction. Here, we use lytic activity to test for trade-offs between immune ability and reproductive events in three experiments with the pholeid spider *Physocyclus dugesi* (Simon 1893). First, we test whether males or females have their immune response negatively affected after mating; second, we assess whether oviposition behavior affects immune response; and third, we investigate whether sexual aggression by females affects immune response. We compare reproductive and non-reproductive spiders' immune response. Our results suggest a down-regulation of immune response following mating, oviposition, and aggression. This supports the notion that immunocompetence is competing for a resource with sexual activities. We discuss reasons why such costs arise in *P. dugesi*.

Keywords: Immune response, mating, oviposition, aggression, trade-off

Parasites are major selective agents such that hosts have evolved a number of adaptations to counter infection. One such adaptation is the immune system, by which hosts recognize self from non-self and act accordingly to eliminate intruders (reviewed by Schmid-Hempel 2011). The immune response imposes both evolutionary and proximate costs (reviewed by Schulenburg et al. 2009; Schmid-Hempel 2011). Given that other life history traits (e.g., traits involved in growth and reproduction) are also costly, infected hosts must face resource allocation dilemmas (Sheldon & Verhulst 1996; Lawniczak et al. 2006). Infections ought to lead to trade-offs where hosts allocate resources to one life history function at the cost of other functions (Schulenburg et al. 2009). The costs of immune responses have been investigated in invertebrates in a variety of contexts with conflicting results.

Researchers of several studies of invertebrates have detected that immune ability may be reduced during or after sexual activity (reviewed by Lawniczak et al. 2006). For example, immune function becomes impaired during copulation (Siva-Jothy et al. 1998; McKean & Nunney 2001; Rolff & Siva-Jothy 2002; Fedorka et al. 2004), oviposition (Siva-Jothy et al. 1998), and male aggressive behavior (Siva-Jothy et al. 1998; Contreras-Garduño et al. 2006). One proximate explanation for decreases in immune ability is that the juvenile hormone directs resources to sexual activities, thereby impairing immune ability (Rolff & Siva-Jothy 2002).

Other studies, however, show no impairment of immune response by sexual activity in invertebrates. For example, immune ability is enhanced during the time of mating in crickets (Shoemaker et al. 2006) and beetles (Valtonen et al. 2010). Individuals may increase investment in immune ability to counter the increased pathogenic risks of mating, such

as sexually transmitted diseases (Knell & Webberley 2004). Recent work in damselflies found no down-regulation of immune ability during mating and oviposition, presumably because changes in juvenile hormone concentrations were not sufficient to induce resource allocations that impaired immune ability (Córdoba-Aguilar et al. 2011). Whatever the explanation, these counterexamples indicate that the assumed tradeoff between immunity and sexual function is far from a generalized pattern.

Here, we investigate whether a trade-off exists between immunocompetence and sexual behavior in the round-bodied daddy long leg spider, Physocyclus dugesi (Simon 1893). Using three independent experiments, we tested whether mating behavior, oviposition, and agonistic interactions between females affected immunocompetence. As a measurement of immunocompetence, we used lytic activity (LA), a key immune ability variable that has been previously used in studies of ecological immunity in spiders (e.g., Ahtianen et al. 2004, 2005, 2006). Measures of LA quantify the digestive action of a wide variety of antimicrobial peptides activated upon infection. LA is specific in action (Bulet 1999; Genta et al. 2003; Wang et al. 2011), and isoforms of many antimicrobial peptides are present as precursors, allowing rapid immune responses following pathogenic invasions (Hetru 1998). The production of antimicrobial peptides is energetically costly (Ahtianen et al. 2005). We predicted that individuals that mate, oviposit, or interact agonistically during sexual encounters will show reduced levels of LA compared to control individuals.

METHODS

Study species.—P. dugesi builds irregular webs that support solitary or group living arrangements of males and females of diverse ages and sizes. Reproductive activity occurs all year

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long, peaking from May to September (Rodríguez-Márquez & Peretti 2010). Mating has a courtship stage that lasts a few minutes, during which both sexes make contact with each other using their legs with vibrating movements along the entire body (Rodríguez-Márquez & Peretti 2010). This is followed by copulation. The entire mating sequence lasts approximately 60 min (Rodríguez-Márquez & Peretti 2010). Fifteen to 20 d following mating, the female lays 10–50 eggs in an egg sac and then holds the egg sac in her chelicerae until the eggs hatch. During this period, females feed infrequently, as otherwise they would have to abandon the egg sac temporarily, which is rarely observed (A. Peretti pers. obs.). Furthermore, egg-sac laden females are aggressive toward other conspecific females that approach (A. Peretti pers. obs.)

Collecting and rearing.—We collected females in their penultimate instar (n = 98) and adult males (n = 38) at the Universidad Nacional Autónoma de México, campus Ciudad Universitaria (19°20'01"N, 99°11'54"W), between April and July 2010. We placed each individual in a plastic container (8 × 12 cm) containing paper (providing a surface for web building) with a wet cotton ball (providing a water source) placed at the base of the container (which was cleaned daily) and maintained these containers at ambient conditions (photoperiod approximately 12/12 h, 11.9-25.5 °C, 57% relative humidity). To collect known virgin females, we maintained juveniles until they reached adulthood. Since experiments were done concurrently, we randomly assigned males and females to specific experiments and treatments. As a measure of body size, we used the tibia-patella length (in mm) of the first pair of legs (cf. Jakob 1994; Huber 1996). For food, we used Drosophila melanogaster adults and Tenebrio monitor larvae, provided ad libitum once every week. Voucher specimens were deposited in the insect collection of the Instituto de Ecología, Universidad Nacional Autónoma de México.

Estimation of LA.—One of the best ways to measure immunocompetence in arthropods is the assessment of LA (Ellis 1990; Rantala & Kortet 2003; Ahtianen et al. 2004). We drew a sample of 3 ul of haemolymph from each individual by severing its first pair of legs and collecting the haemolymph with a sterile capillary tube. Subsequently, all individuals were stored in 70% ethanol for further measures of body size. Following the technique used by Ahtianen et al. (2004, 2005), each haemolymph sample was mixed with 20 ul buffer (PBS, 0.067 M phosphate, 0.9% NaCl, pH 6.4) and frozen at -80°C . After thawing samples, we pipetted them into an ELISA plate. PBS buffer was used as a negative control. Samples and controls were mixed with 80 ul of a suspension that contained 0.0002 mg/ml of bacteria (liophilized *Micrococcus*, Sigma). We then measured optical density at 492 nm at room temperature in intervals of 1 min. LA was expressed as changes in optical density of a sample after an interval of 10 min; the higher the optical density reading the lower the LA.

Experiments.—Mating and LA: We had two groups, experimental (mated animals) and control (unmated animals), to which individuals were assigned randomly. For each mating trial, a virgin female that had just reached adulthood was placed in a larger plastic container (10 × 15 cm, thus facilitating the observer's detection of mating) 24 h before the male, so that the female was able to build the web on which they would copulate. Then we introduced the male.

After the male was placed in the container with the female, mating typically commenced after approximately 20 min. There were instances during which neither the male (6 out of 10 cases) nor the female (4 out of 8 trials) were interested in mating. When individuals were unresponsive for 20 min, the disinterested male was replaced by a new individual. All individuals exposed to a mating trial were removed from the experiments, regardless of whether or not they actually engaged in reproductive behavior. Unmated individuals (10 females and 10 males) were treated as mated animals but were never allowed to mate. Females were also introduced to the plastic container indicated above, but no male was introduced, and males were not exposed to any female. Both mated and unmated males were used within 15 d after their capture to reduce effects of potential differences of recent mating histories [for example, a recent mating may affect an individual's immune state, which can be recovered after a few days: for a review in arthropods, see Lawniczak et al. (2006)]. There was no difference in the median sizes of individuals assigned to each treatment (mated males, median 12.709 mm, range 12.132–13.509, vs. unmated males, median 13.24 mm, range 11.711-15.213, Mann Whitney test = -1.109, P = 0.866; mated females, median 10.86 mm, range 10.149-11.982, vs. unmated females, median 11.594 mm, range 10.733-12.147, Mann Whitney test = 0.139, P = 0.448). Each mating lasted exactly 60 min, after which both male and female were removed for immediate haemolymph extraction and LA determination (see above). LA was compared between males that had mated (n = 4) and males that had not mated (control males, n = 10), and between females that had mated (n = 4) and females that had not mated (control females, n = 10).

Oviposition and LA: We selected 10 females collected as adults and placed them in containers (10×15 cm). As indicated above, oviposition in these species occurs after approximately 15 d. The day after oviposition we removed the egg sacs and took haemolymph samples from each female. We tested for an effect of oviposition by comparing LA of these females to a control group (n = 10) of known virgin females (collected in their penultimate instars and kept until mature, which was 5 d after they reached adulthood. Size did not differ between these groups (females that oviposited, median 10.967, range 10.119–12.493; females that did not oviposit, median 11.594, range 10.733–12.147; Mann Whitney test = -0.267, P = 0.605).

Agonistic interactions and LA: Agonistic interactions were staged between virgin adult females, which we kept in similar conditions prior to the experiment. One day after reaching adulthood each unmated adult female was placed in a plastic container of 10×15 cm for 5 d. Then, one spider (intruder) was removed from its container and placed inside a container that already had a female (resident). We assigned intruder or resident roles at random. We then observed animals continuously for 2 h. Aggression was defined as contact made with any of the legs of both spiders. Aggressive behavior was shown by both spiders in all trials (n = 10). Immediately after each trial, we extracted haemolymph from both females and compared LA of residents (n = 10), intruders (n = 10), and a control group whose females were never exposed to any interaction (n = 10). Residents (median 10.793, range 9.952–



Figure 1.—Optical density readings (of bacterial suspension) according to mating status for both sexes. Each box plot indicates the median \pm one quartile; the whiskers show the data range.

11.123), intruders (median 11.227, range 10.19–13.007), and control females (median 11.594, range 10.733–12.147) did not differ in body size (Kruskal Wallis = 2.182, P = 0.336).

Statistical analyses.—Due to the small sample sizes in each group, we used Mann Whitney tests to test for LA differences among treatment groups in each experiment. All immune values are given as optical density. We used NCSS 2007 for statistical analysis.

RESULTS

Mating and LA.—Mating was associated with lower lytic activity. Mated males (median 0.935, range 0.929–0.980) showed higher levels of optical density than non-mated males (median 0.891; range 0.875–0.905, Mann Whitney test = 2.466, P = 0.006), thus indicating that mated males had lower lytic activity. Similarly, mated females (median 0.980, range 0.929–1.003) showed higher levels in optical density than non-mated females (median 0.849, range 0.840–0.868, Mann Whitney test = 2.557, P = 0.005; Fig. 1).

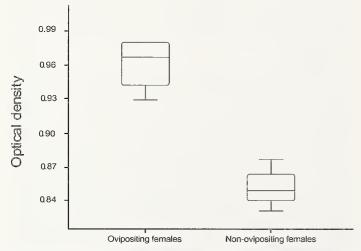


Figure 2.—Optical density readings (of bacterial suspension) in females according to whether they oviposited or not. Each box plot indicates the median \pm one quartile; the whiskers show the data range.

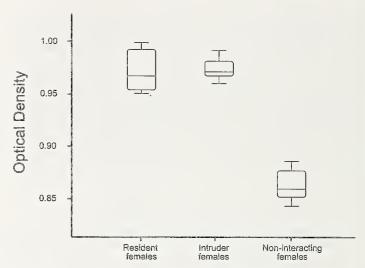


Figure 3.—Optical density readings (of bacterial suspension) in resident, intruder and non-interacting females. Each box plot indicates the median \pm one quartile; the whiskers show the data range.

Oviposition and LA.—Oviposition was associated with lower lytic activity. Females that oviposited (median 0.967, range 0.935–0.981) showed higher levels of optical density than females that did not oviposit (median 0.849, range 0.840–0.868, Mann Whitney test = 3.298, P = 0.0005: Fig. 2).

Agonistic interactions and LA.—There was no difference in optical density of LA activity between resident (median 0.953, range 0.943–1.037) and intruder females (median 0.962, range 0.957–0.981, Mann Whitney test = 3.738, P=0.354). Given this, we pooled both resident and intruder females and compared them against control females to see whether interacting females (resident and intruder) showed lower levels of LA than non-interacting females (control). Interacting females (median 0.962, range 0.952–0.988) had higher levels of optical density than control females (median 0.849, range 0.840–0.868, Mann Whitney test = 3.722, P < 0.001) (Fig. 3).

DISCUSSION

The results of empirical studies are mixed with regard to whether sexual activities such as mating, oviposition, and aggression impair immune ability in invertebrates (reviewed by Córdoba-Aguilar et al. 2011). Despite our small sample size, our results support the hypothesis of a trade-off between immune ability and reproduction and between immune ability and aggressive interactions. Below, we examine the potential causes behind this relationship in terms of resource allocation between immune and sexual functions and the down-regulation of immunocompetence.

Mating reduces immunocompetence in *Physocyclus dugesi*, but such trade-offs may have different explanations in each sex. In males, the energetic cost originates from the behavior males perform during mating, such as movements of the pedipalps, vibrations of the abdomen and leg vibrations (Huber & Eberhard 1997) and intense twisting and squeezing movements using both pedipalps within the female's genital opening (Rodríguez-Márquez & Peretti 2010; Huber 1995; Kaster & Jakob 1997). In contrast, only some vibrations appear to originate from the female during mating, as sometimes females tapped briefly with their anterior legs

(Huber & Eberhard 1997). Thus the negative correlation between immunocompetence and reproduction may not be related to behavior in females.

The immunocompetence cost of mating in females may come from a down-regulation to save energetic resources for the future expenditures of oviposition. For example, it may be that females need to find a particular place for oviposition or use specific resources for egg provisioning that may be energetically demanding. Furthermore, as has been demonstrated in other invertebrates, males could transfer seminal products during copulation, which may induce female diversion of resources to egg production at the expense of female immunity (reviewed in Lawniczak et al. 2006).

In relation to the cost of oviposition, females may face resource allocation dilemmas during this period so that immune ability is again compromised. Reductions in LA levels, however, may be alternatively explained by mechanisms apart from energetic demands (e.g., Siva-Jothy et al. 1998). In some insects, juvenile hormone down-regulates immune ability during mating and oviposition (Rolff & Siva-Jothy 2002), although there are important exceptions to this (e.g., Córdoba-Aguilar et al. 2011).

Agonistic interactions also led to reduced immunocompetence, as found in other arthropods (e.g., Contreras-Garduño et al. 2006, 2009). In pholcids, agonistic interactions are common and could elicit costs in at least three different situations (Jakob 1999). One first cost is due to reduced access to food. For example, Jakob (1991) found in Holocnemus pluchei (Scopoldi 1763) that gregarious individuals fed less than solitary individuals. A second context is related to the cost of being injured or cannibalized in aggressive interactions with conspecifics. The final context is the cost of web site and or web investment. Such a cost will emerge if, for example, a spider is driven away from its own web and needs to find another web or build a new one (Jakob 1999). As a complement to the immunocompetence costs measured here, future studies should seek to quantify the energetic resources spent during aggression. One likely variable appropriate for such an investigation is muscular lipid-based fat, as in other insects (Contreras-Garduño et al. 2006).

Despite our support for the negative effect of reproductive activities on immunocompetence, other studies have found contrary results (e.g., Shoemaker et al. 2006; Valtonen et al. 2010; Córdoba-Aguilar et al. 2011). There are different explanations for such disparate findings. One is that the difference may be due to the particular biology of some species. For example, Valtonen et al. (2010) documented that mating enhanced resistance against fungal infections in the mealworm beetle, Tenebrio molitor, a species whose males and females are highly promiscuous (e.g., Eady 1995). A second explanation is that the condition of the animal may play a role at the time reproductive activities take place. It is known that resource allocation conflicts, when immunity is involved, will be more dramatic for animals in poor condition than for animals in good condition (Sheldon & Verhulst 1996). Related to this, studies carried out under field and laboratory conditions may have used animals that differed widely in condition. Even in the laboratory, differences in condition may be found. If food is provided, animals may eat more to compensate for the energetic demands imposed by immuno-

logical costs (e.g., Povey et al. 2009), a situation that is usually not controlled. One final explanation is that the immunological cost exists, but that finding it depends on the immune parameter being used. This is because it is known that not all immune parameters may indicate animal condition or resistance (e.g., Adamo 2004). In fact, a number of arthropod studies have used phenoloxidase activity, a key effector during immune response (reviewed by González-Santoyo & Córdoba-Aguilar 2012), for detecting immunological costs of different energetically demanding activities that include reproductive activities (reviewed by Lawniczak et al. 2006). Paradoxically, a recent review concluded that phenoloxidase activity does not indicate the host's resistance and only under some circumstances correlates with the host's condition (reviewed by González-Santoyo & Córdoba-Aguilar 2012). Given the above potential sources of noise, we concur with Valtonen et al. (2010) that it is still premature to conclude that reproductive activities impair immunocompetence in arthropods.

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Fine tuning of vision-based prey-choice decisions by a predator that targets malaria vectors

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Abstract. Evarcha culicivora Wesolowska & Jaekson 2003 is a jumping spider (Aranea: Salticidae) that has the distinction of being the only predator known to express an active preference for the vectors of human malaria (i.e., the mosquito genus Anopheles) and to feed indirectly on blood by choosing blood-carrying female mosquitoes as prey. Here we examine this predator's preference profile in greater detail than has been achieved before. Lures (dead prey mounted in life-like posture) were made from two mosquitoes (Anopheles gambiae and Culex quinquefasciatus) and a non-biting midge (Clinotanypus claripennis). Testing protocols were simultaneous presentation (two prey presented simultaneously), alternate day (two prey, each presented singly but on alternate days) and alternative prey (second prey presented while test spider feeding on first prey). Pre-trial fasts were 1, 7, 15 and 21 days. Findings from this combination of variables were used to estimate strengths of preferences. Besides confirming the preference of E. culicivora for blood meals and for Anopheles in particular, we provide the first evidence of a preference, independent of blood meals, for female instead of male mosquitoes. The strength of preference, measured by its persistence despite increasingly long pre-trial fasts, shows that preference for Anopheles is expressed by juveniles more strongly than by adults, but preference for blood meals is expressed by adults more strongly than by juveniles.

Keywords: Foraging, mosquito, predation, prey preference, salticid, specialization

The term 'preference' acknowledges that predators favor certain kinds of prey, while 'choice' refers to behavior that is driven by preference. Data on a predator's natural diet may suggest hypotheses about preferences, and these hypotheses can be used to predict the choices a predator will make. However, data on diet alone cannot reveal a predator's choices and preferences (Huseynov et al. 2008; Nelson & Jackson 2011). Predators that have been shown with appropriate experimental evidence to express distinctive preferences are of particular interest, not only as model organisms for testing predictions derived from foraging theory, but also for research on the mechanisms underlying perception and decision-making. Owing to their ability to see fine detail (Harland et al. 2011) and their intricate vision-guided prey-capture behavior (Jackson & Pollard 1996), jumping spiders (Salticidae) are exceptionally suitable subjects for experimental studies related to predatory preferences.

Striking examples of prey-choice behavior are known (Nelson & Jackson 2011) especially for salticids that prefer other spiders as prey (araneophagic species) and for salticids that prefer ants as prey (myrmecophagic species). However, Evarcha culicivora Wesolowska & Jackson 2003 has the most specific preferences known for a salticid. All ages of this East African species feed indirectly on vertebrate blood by actively choosing as preferred prey blood-fed female mosquitoes, both in a laboratory setting and in nature (Wesolowska & Jackson 2003; Jackson et al. 2005). Evarcha culicivora also distinguishes between mosquito genera, expressing a preference for Anopheles in particular (Nelson & Jaekson 2006). Yet research on the preferences of E. culicivora has been based on only a few of the experimental protocols and pre-trial fasting periods that have been routine in research on araneophagic and myrmecophagic species.

Prey preference of myrmecophagic and areneophagic salticids has been studied using three different protocols and pretrial fasting durations (Li & Jackson 1996; Li et al. 1996, 1997,

1999; Jackson et al. 1998). Here, we apply all three protocols to identify the prey preferences of *Evarcha culicivora* and to better understand the strength of its preferences.

Of these three protocols, alternative-prey testing can be envisaged as requiring the strongest expression of preference because, in these tests, a salticid is already feeding when offered a different kind of prey. Consequently, to express a preference, a spider must release an already secured prey to capture an alternative. With simultaneous-presentation testing, a salticid is given access to two potential prey at the same time and is allowed to make a snap decision to choose one or the other. Alternate-day testing, where a spider's inclination to take each of two prey types is assessed in isolation from the other, can be envisaged as a method for discerning preference stronger than that required by simultaneous-presentation testing, but not requiring the strength of preference required by alternative-prey testing. With all three testing protocols, how fasting affects preference is also of interest because one of the most basic predictions from foraging theory is that predators will become less selective in times of prey scarcity (see Sih & Christensen 2001). This, in turn, is a rationale for predicting that longer fasts will be necessary before stronger preferences dissipate.

The alternate-day and alternative-prey protocols, and the longer pre-trial fasting durations used in experiments on myrmecophagic and araneophagic salticids, have not been used previously in research on *E. culicivora*. Yet, with the preference of *E. culicivora* appearing to be especially complex, this is a species for which data from the full range of testing protocols and fasting duration would be of particular interest. Here we consider the relative strengths of preferences for blood meals and also for *Anopheles* when neither choice had received a blood meal. We compare the preference profiles of adult females with those of juveniles of *E. culicivora*. We also investigate, for the first time, the hypothesis that *E. culicivora*, independent of blood meals, expresses a preference for female

rather than male mosquitoes, based on visual cues alone. In mosquitoes the major morphological difference between the sexes lies in the number of setae on the antennae, with male antennae appearing more plumose than those of females. The rationale for our hypothesis is the fact that males subsist primarily on nectar (Klowden 1995), while only female mosquitoes feed on blood (Clements 1999). For the spider, choosing female mosquitoes will not suffice for acquiring a blood meal. However, taking an interest in female mosquitoes, independent of whether she is seen to be carrying blood, might be advantageous in the context of predisposing *E. culicivora* to pay attention to the mosquitoes that have the potential of being blood carriers.

METHODS

Spiders used for tests were juveniles (2.0 mm) and adult females (5.5 mm) of Evarcha culicivora (body length accurate to nearest 0.5 mm). All test spiders were from laboratory culture (see Jackson et al. 2005), with rearing diet consisting of lake flies (i.e., non-biting midges, known locally as 'lake flies': Chironomidae) and blood-fed female mosquitoes (Culex quinquefasciatus and Anopheles gambiae s.s.; henceforth simply Culex and Anopheles, respectively) provided ad libitum three days a week. Lake flies were collected locally as needed, and mosquitoes came from laboratory culture (see Jackson et al. 2005). In culture, all mosquitoes had continuous access to glucose (6% solution). Mosquitoes used for making lures were both sexes of Culex and Anopheles. Two types of female mosquitoes were used: 'blood-fed' (received blood meal 4 h before feeding spiders or being used for making lures) or 'sugar-fed' (no blood meals). Females of Clinotanypus claripennis, a common chironomid in our field site, were also used for making lures. For standardization, all insects used for making a lure were 5.0 mm in body length (accurate to the nearest 0.5 mm). Each insect was mounted centered on a cork disc and then sprayed with a transparent plastic adhesive which served to preserve lures and prevent odor cues from affecting test outcome (for details concerning making lures, see Jackson et al. 2005).

The testing arena (walls 35 mm high, see Fig. 1 for other dimensions) was a glass box with a removable glass lid that sat centered on top of a 150 mm high Plexiglas stand. All testing was carried out between 0800 and 1400 hours (laboratory photoperiod 12L:12D, lights on at 0700 hours). No test spider or lure was used in more than one type of test. We introduced spiders into the arena through a hole in the floor (Fig. 1). This hole, situated with its closer side 10 mm from one end of the box, was plugged with a removable rubber bung.

At the opposite end of the arena, there was a 'left lure hole', a 'right lure hole' and a 'central hole' (diameter of each, 5 mm). Lures were positioned outside the arena so that spiders could only see them through the arena's glass walls, and thus could not detect any odor cues. In simultaneous-presentation tests, a lure was centered on top of the right hole, and another lure was centered on the top of the left hole. In alternate-day and alternative-prey tests, a single lure was centered on the top of the central hole. The lure was placed such that it faced directly toward the side of the arena. The lure stayed in place because the diameter of the hole in the stand was narrower than the diameter of cork disc holding the lure.

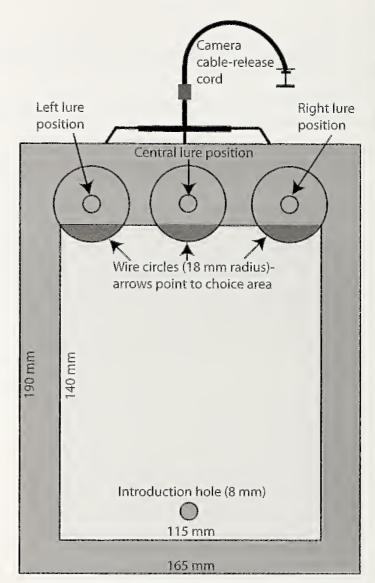


Figure 1.—Testing apparatus. Rectangular glass box (white rectangle in figure) with glass lid, sitting on top of Plexiglas stand (grey rectangle). Moving lures controlled by using a camera release cord. 'Choice area': dark grey semicircular area within wire circles. Left and right lure positions used in simultaneous-presentation tests. Central lure position used in alternate-day and alternative-prey tests.

A metal prong attached to a camera cable-release cord was connected to the underside of each of the two cork discs in simultaneous-presentation tests and to the single cork disc in alternate-day and alternative-prey tests. Pressing the cablerelease moved each lure 5 mm above the floor of the arena and then, by releasing the cable, each lure moved back to the floor. As soon as the test spider entered the arena, the cable-release was pressed once every 30 s and then released immediately, causing the lure/s to move up once and down one time for each press. This ensured that salticids remained interested in the lures, as motionless prey are less effective at eliciting responses from salticids. In each simultaneous-presentation test, there were two lure types. In alternate-day and alternative-prey tests, the single lure that was present on one day was different from the single lure that was present on the other day in the pair of tests.

Table 1.—Alternative-prey testing (see text for details) of *Evarcha culicivora* adults (data outside parentheses) and juveniles (data inside parentheses). Prey presented as moving lures: *Clinotanypus claripennis* (Cc), blood-fed *Anopheles* female (Af), sugar-fed *Anopheles* female (Afs), *Anopheles* male (Am), blood-fed *Culex* female (Cf), sugar-fed *Culex* female (Cfs), *Culex* male (Cm). Note: most spiders did not drop any prey for the other (N paired tests minus sum of both columns depicting numbers of prey that were dropped). ¹ Depicts one day fasted. ⁷ Depicts seven days fasted. * P < 0.05.

Prey 1	Prey 2	Dropped prey 2 for prey 1	Dropped prey 1 for prey 2	N paired tests
¹ Af	Am	13* (2)	0 (1)	40 (35)
⁷ Af	Am	1 (1)	0 (1)	40 (30)
¹ Af	Afs	11* (0)	1 (0)	41 (25)
⁷ Af	Afs	0 (0)	0 (1)	30 (28)
¹ Af	Cf	0 (0)	0 (0)	40 (50)
⁷ Af	Cf	0 (0)	0 (0)	40 (50)
¹ Af	Cfs	11* (0)	1 (0)	40 (30)
⁷ Af	Cfs	0 (0)	0 (0)	40 (30)
¹ Af	Cc	13* (0)	0 (0)	60 (25)
⁷ Af	Cc	0 (0)	0 (0)	50 (29)
¹ Cf	Cm	10* (0)	0 (0)	50 (40)
⁷ Cf	Cm	0 (0)	0 (0)	50 (40)
¹ Cf	Cfs	8* (0)	0 (0)	31 (25)
⁷ Cf	Cfs	0 (0)	0 (0)	35 (25)
¹ Cf	Afs	12* (0)	2 (0)	60 (40)
⁷ Cf	Afs	0 (0)	0 (0)	50 (40)
¹ Cf	Ce	9* (1)	0 (0)	40 (25)
⁷ Cf	Ce	0 (0)	0 (0)	35 (20)
¹ Afs	Cfs	0 (0)	1 (0)	40 (40)
⁷ Afs	Cfs	0 (0)	0 (0)	40 (40)
¹ Afs	Am	1 (0)	0 (0)	40 (40)
⁷ Afs	Am	0 (0)	0 (0)	40 (40)
¹ Afs	Cm	0 (0)	1 (0)	57 (25)
⁷ Afs	Cm	0 (0)	0 (0)	40 (25)
¹ Afs	Cc	0 (0)	0 (0)	40 (30)
⁷ Afs	Cc	0 (0)	0 (0)	40 (30)
¹ Am	Cm	0 (0)	0 (0)	35 (40)
⁷ Am	Cm	0 (0)	1 (0)	35 (40)
¹ Am	Cc	0 (0)	0 (0)	35 (25)
⁷ Am	Ce	0 (0)	0 (0)	30 (20)
¹ Cm	Cc	0 (0)	0 (0)	35 (20)
⁷ Cm	Ce	0 (0)	0 (0)	35 (20)
¹ Cfs	Cm	0 (0)	0 (0)	30 (23)
⁷ Cfs	Cm	0 (0)	0 (0)	30 (25)
¹ Cfs	Am	0 (0)	0 (0)	25 (20)
⁷ Cfs	Am	0 (0)	0 (0)	25 (22)
¹ Cfs	Cc	0 (0)	0 (0)	40 (40)
⁷ Cfs	Cc	0 (0)	0 (0)	40 (40)

Two circles made from thin copper wire were situated on the platform in simultaneous-presentation tests, and one circle made from thin copper wire was situated on the platform in alternate-day and alternative-prey tests. A lure hole was at the center of each circle, and a part of each wire circle extended under the arena (Fig. 1), visible because the bottom of the arena was made of glass. The part of the circle under the arena was the 'choice area' (Fig. 1). Our operational definition of a choice was seeing the test spider fixate its gaze on a lure and then, while retaining fixation, entering the choice area. 'Fixate' refers to the corneal lenses of the salticid's large forward-facing principal eyes being held oriented toward a lure. There were rare instances (< 5%) of the 15-min test period ending with the test spider outside the choice area, but with its gaze fixated on a lure. In these instances, we extended the test period until the test spider either made its choice or turned away.

During simultaneous-presentation testing, the test spider was exposed to two lures at the same time, each being made from a different kind of prey (side for each lure determined at random). These data were analyzed using chi-square tests of goodness of fit. In alternate-day tests, the test spider was exposed to a single lure of one type on one day and a single lure of another type on the next day (which prey type presented first randomized). In alternate day tests, only those test pairs in which E. culicivora chose one prey, but not the other, provided evidence of preference. In alternative-prey tests, the spider was exposed to a single lure made from one prey type while feeding on another prey type. Alternativeprey testing, like alternate-day testing, was carried out in two trials, one on each of two successive days. The prey type on which the test spider was feeding on the first day was the prey type provided as a lure on the second day. Which of the two

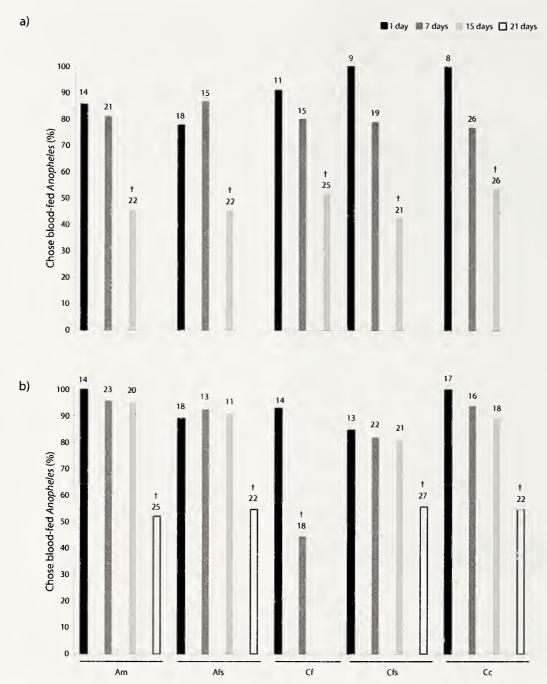


Figure 2.—Expression of preference during simultaneous-presentation tests (data analysis: test of goodness of fit, $H_o = 50/50$) by a) juveniles and b) adults of *Evarcha culicivora*. Pre-trial fast durations indicated by different shadings. Each spider tested with blood-fed *Anopheles* female and with another prey. Data expressed as percentage of test spiders that chose *Anopheles* females (N used for statistics over bar). Other prey: *Anopheles* male (Am), sugar-fed *Anopheles* female (Afs), blood-fed *Culex* female (Cf), sugar-fed *Culex* female (Cfs), *Clinotanypus claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed *Anopheles* (P < 0.05).

prey types served as a lure in the first-day trials was randomized.

Each simultaneous-presentation and alternate-day test began when the test spider entered the arena. However, before alternative-prey tests were initiated, the test spider was put in a Petri dish (diameter 90 mm) with a single prey item of one type. The test spider usually captured and began feeding on this prey no later than 15 min after being introduced into the Petri dish, and 30 s after beginning to feed the test spider was introduced, while still feeding, into the arena. Testing was

cancelled on the rare occasions when the test spider failed to capture the prey in the Petri dish within 15 min or failed to hold on to the prey when being introduced into the arena. These spiders were not used again. The operational definition of 'choice' in alternative-prey testing included an additional requirement: the spider had to drop the prey on which it was feeding, either before entering or while inside the choice area, and only spiders that dropped their prey were used for analysis. However, no spiders entered the choice area carrying prey and left it still carrying the prey, nor were there any

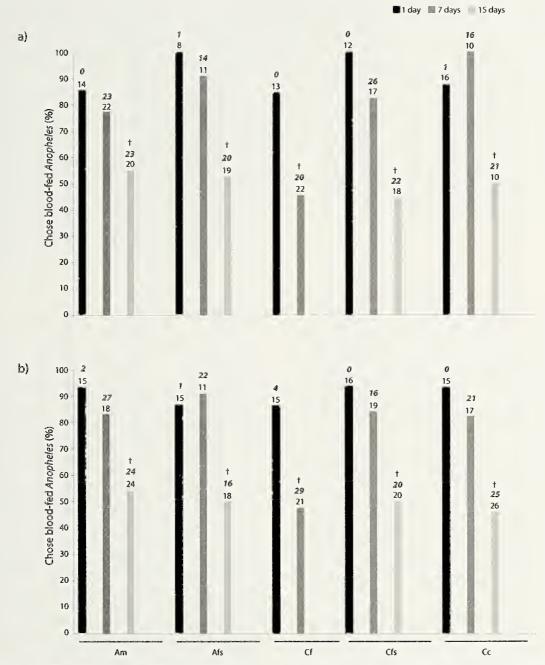


Figure 3.—Expression of preference during alternate-day tests (data analysis: McNemar tests for significance of changes) by a) juveniles and b) adults of E. culicivora. Pre-trial fast durations indicated by different shadings. Each spider tested with blood-fed Anopheles female and with another prey. Data plotted include only results from spiders that moved to the choice area for one prey and not the other (i.e., those that chose neither or both prey were omitted). Data expressed as percentage of test spiders that chose Anopheles females (N used for statistics over bar). Other prey: Anopheles male (Am), sugar-fed Anopheles female (Afs), blood-fed Culex female (Cf), sugar-fed Culex female (Cfs), C. claripennis (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed Anopheles (P < 0.05). The number of spiders that chose both prey is indicated in bold italics above bars.

instances of spiders outside the choice area dropping their prey without being fixated on a lure.

Four feeding regimes (pre-trial fasting durations) were adopted: 1, 7, 15 or 21 days (i.e., the test spider was fed to satiation, held without prey for the indicated fast duration and then tested). Two of these feeding regimes (1-day and 7-day fast) were adopted in all experiments. Whenever preference was still evident after a 7-day fast, testing was also carried out

after a 15-day fast and, whenever preference was still evident after a 15-day fast, testing resumed after a 21-day fast.

When used in alternate-day and alternative-prey testing, the test spider was exposed to a moving lure on two successive days and this meant that, on the second day, fasting duration was actually a day longer than stated (e.g., '7-day fast' means the test spider's last meal was 7 days before the first and 8 days before the second test in the test pair). The data analysis used

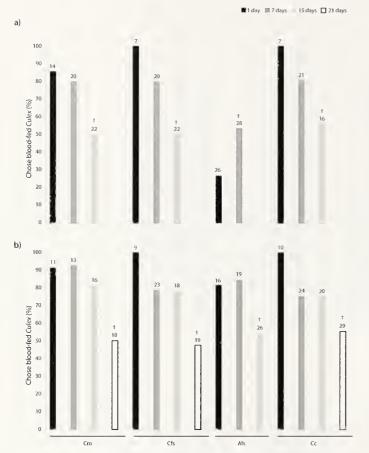


Figure 4.—Expression of preference during simultaneous-presentation tests (data analysis: test of goodness of fit, $H_o = 50/50$) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with blood-fed *Culex* female and with another prey. Data expressed as percentage of test spiders that chose *Culex* females (N used for statistics over bar). Other prey: *Culex* male (Cm), sugar-fed *Culex* female (Cfs), sugar-fed *Anopheles* female (Afs), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed *Culex* (P < 0.05).

(McNemar tests for significance of changes) considers only the instances in which a test spider chose a lure on one, but not the other, of the two successive days. However, for spiders subjected to a 1-day fast, we ensured that the fast was 1 day for the first and for the second test of the test pair. This was achieved by feeding the test spider to satiation immediately after testing on the first day. The stringency of alternative prey testing made obtaining these data particularly difficult (because test spiders rarely dropped one prey to take another), leaving us with only small sample sizes for statistical analysis despite the numerous tests carried out (20 to 60 paired tests, see Table 1).

For each pair of prey types, we calculated an arbitrary preference index, this being a number that increased with the number of testing protocols and fasting durations for which significant evidence of preference had been shown. For example, if a blood-fed female *Anopheles* was chosen over a particular alternative under all three testing protocols, but not after fasts longer than 7 days (second of 4 possible fasting durations), this would have an index of $3 \times 2 = 6$.

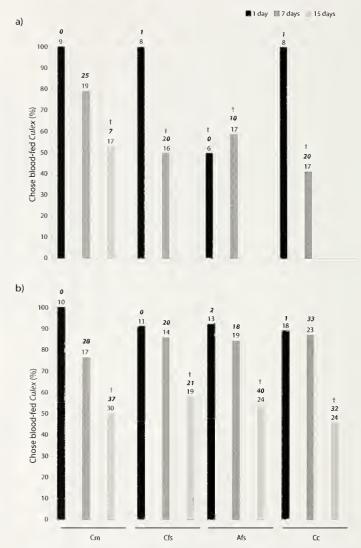


Figure 5.—Expression of preference during alternate-day tests (data analysis: McNemar tests for significance of changes) by a) juveniles and b) adults of E. culicivora. Pre-trial fast durations indicated by different shadings. Each individual spider tested with blood-fed Culex female and with another prey. Data plotted include only results from spiders that moved to the choice area for one prey and not the other (i.e., those that chose neither, or both prey were omitted). Data expressed as percentage of test spiders that chose Culex females (N used for statistics over bar). Other prey: Culex male (Cm), sugar-fed Culex female (Cfs), sugar-fed Anopheles female (Afs), C. claripennis (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose blood-fed Culex (P < 0.05). The number of spiders that chose both prey is indicated in bold italics above bars.

Voucher specimens of all species have been deposited in the Florida State Collection of Arthropods, Gainesville, Florida, USA.

RESULTS

Preference for blood.—First, we consider data from adult test spiders. In all instances and regardless of testing method, when one prey was a blood-fed female mosquito and the other was not carrying blood (i.e., the other prey was a sugar-fed female mosquito, a male mosquito, or a lake fly), preference

Table 2.—Preference indices for juveniles and adults of *Evarcha culicivora*. Preference index derived by multiplying the number of testing protocols under which significant evidence of preference was shown times the fasting durations under which significant evidence of preference was shown (see text for example). Higher values for the index indicate stronger preferences. With one exception*, preference is for prey 1. * Chose prey 2 (sugar-fed *Anopheles* female).

Prey 1	Prey 2	Juvenile index	Adult index
Blood-fed Anopheles female	Anopheles male	4	6
Blood-fed Anopheles female	Sugar-fed Anopheles female	4	6
Blood-fed Anopheles female	Blood-fed Culex female	3	2
Blood-fed Anopheles female	Sugar-fed Culex female	4	6
Blood-fed Anopheles female	Lake fly	4	6
Blood-fed Culex female	Culex male	4	6
Blood-fed Culex female	Sugar fed Culex female	3	6
Blood-fed Culex female	Sugar-fed Anopheles female	1*	5
Blood-fed 'Culex female	Lake fly	3	5
Sugar-fed Anopheles female	Sugar-fed Culex female	4	2
Sugar-fed Anopheles female	Anopheles male	3	2
Sugar-fed Anopheles female	Culex male	3	2
Sugar-fed Anopheles female	Lake fly	3	2
Sugar-fed Culex female	Culex male	0	0
Sugar-fed Culex female	Anopheles male	0	0
Sugar-fed Culex female	Lake fly	0	0
Anopheles male	Culex male	3	0
Anopheles male	Lake fly	1	0
Culex male	Lake fly	0	0

for the blood meal was expressed (Table 1, Figs. 2–5). However, there was variation in the fasting durations over which preferences were maintained (Figs. 2–5). With two exceptions, the preference index (see Table 2) was 6, the exceptions being a slightly lower preference index of 5 when blood-fed *Culex* females were paired with sugar-fed *Anopheles* females or with lake flies.

Next we consider data from juveniles. When the blood meal was a *Culex* female and the no-blood meal was an *Anopheles* female (i.e., when preference for *Anopheles* clashed with preference for blood meals), preference for *Anopheles* was expressed in preference to blood (Fig. 4). However, as the expression of this preference dissipated after a 7-day fast, the preference index was only 1. In all other instances of simultaneous-presentation and alternate-day testing, preference for blood meals was expressed, but the fasting durations over which preference was maintained varied (Figs. 2–5), and no preference was expressed in alternative prey testing (Table 1). When the blood meal was an *Anopheles* female, the preference index was typically 4, which was slightly higher than the preference index characteristically expressed for blood-fed *Culex* females (Table 2).

Preference for *Anopheles*.—Except in alternative-prey tests (Table 1), adult and juvenile test spiders expressed preference for *Anopheles* when both prey were blood-fed mosquitoes (adult index 2, juvenile index 3; Figs. 2, 3) and when both were sugar-fed mosquitoes (adult index 2, juvenile index 4; Figs. 6, 7). When the two mosquitoes were males, preference for *Anopheles* was expressed by juveniles (index 3), but not by adults (Fig. 8, Table 2).

Preference for female mosquitoes.—When tested with two mosquitoes, one being a male and the other being a female, with neither carrying blood, preference for the female was expressed when the female was *Anopheles* (Figs. 6, 7), regardless of whether the male was *Anopheles* or *Culex* (for both mosquito species: preference index was 2 for adults and 3 for juveniles). However, no preference was evident when the

female was *Culex* (Fig. 9), regardless of whether the male was *Anopheles* or *Culex* (Table 2).

Preference for mosquitoes when the alternatives are lake flies.—When one prey was a lake fly and the other was a mosquito that was not carrying blood, adults expressed preference for *Anopheles* females (Figs. 6, 7; preference index 2), but not for *Culex* females (Fig. 9) and, with the exception of juveniles that had fasted for only 1 day in simultaneous-presentation tests (Fig. 8), not for males of either mosquito species (Table 2). Adult and juvenile spiders expressed preference (Table 2) for *Anopheles* females (Figs. 6, 7), and juveniles expressed a weak preference for males (Fig. 9), but neither juveniles nor adults expressed preference for either sex of *Culex* when tested against lake flies (Figs. 8, 9).

Response levels.—There was a significant effect of fasting time on the number of juveniles ($H_2 = 36.34$, P < 0.001) and adults (Kruskal-Wallis, $H_3 = 35.33$, P < 0.001) that responded in simultaneous-presentation tests, as well as in alternate-day tests (juveniles, $H_2 = 27.18$, P < 0.001; adults, $H_2 = 33.73$, P < 0.001). In both cases, the effect was based on a sharp drop in the number of spiders that failed to choose between 1 and 7-day fasts (Fig. 10).

In alternative prey tests spiders did not respond at all beyond 7-day fasting periods (no spiders ever dropped their prey for the alternative), so instead of using Kruskal-Wallis tests, these data were analyzed using Mann-Whitney U tests for 1-day and 7-day fasting data. However, the number of juveniles (U = 178.5, P = 0.964) and the number of adults (U = 147.5, P = 0.335) that failed to choose (i.e., did not drop one prey for the other) did not differ after a 1 or a 7-day fast and instead remained consistently high (Table 1, Fig. 10).

DISCUSSION

The choices made by *Evarcha culicivora* in these experiments reveal the prey preferences of this unusual predator. The prey-preference profile of *E. culicivora* appears to be structured in

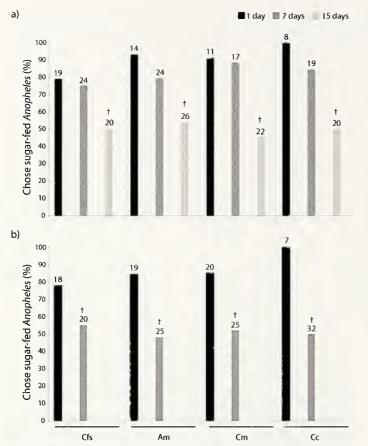


Figure 6.—Expression of preference during simultaneous-presentation tests (data analysis: test of goodness of fit, $H_o = 50/50$) by a) juveniles and b) adults of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with sugar-fed *Anopheles* female and with another prey. Data expressed as percentage of test spiders that chose *Anopheles* females (N used for statistics over bar). Other prey: sugar-fed *Culex* female (Cfs), *Anopheles* male (Am), *Culex* male (Cm), *C. claripennis* (Cc). † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose sugar-fed *Anopheles* (P < 0.05).

tiers of prey choice decisions. This is more complex than the way spider preferences for prey are normally envisaged, and unusual for predators in general. With each of the three testing protocols, we always reached a fasting duration after which preference was no longer detected, but the required duration varied with testing protocol and with whether the spider was an adult or a juvenile. Juveniles and adults expressed preferences for the same prey types, but there were differences in strength and priority. When test spiders were adults, the highest priority was consistently for blood meals, with a preference for Anopheles independent of acquiring a blood meal seeming to be superimposed as a secondary preference. These findings, based on more testing protocols and fasting durations than previously used, confirm basic conclusions from earlier studies (Jackson et al. 2005; Nelson & Jackson 2006).

We found that preference for *Anopheles* over *Culex* is stronger in juveniles than in adults. It is particularly notable that, independent of blood, juveniles expressed a preference for *Anopheles* over *Culex* that was sustained through longer

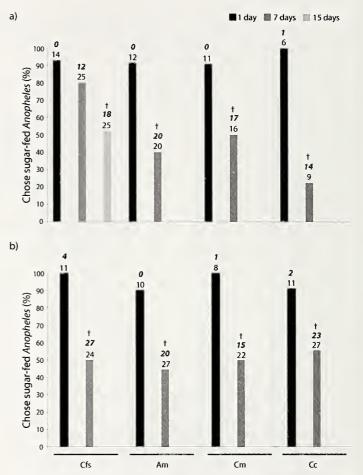


Figure 7.—Expression of preference during alternate-day tests (data analysis: McNemar tests for significance of changes) by a) juveniles and b) adults of $E.\ culicivora$. Pre-trial fast durations indicated by different shadings. Each individual spider tested with sugar-fed Anopheles female and with another prey. Data plotted include only results from spiders that moved to the choice area for one prey and not the other (i.e., those that chose neither or both prey were omitted). Data expressed as percentage of test spiders that chose Anopheles females (N used for statistics over bar). Other prey: sugarfed Culex female (Cfs), Anopheles male (Am), Culex male (Cm), $C.\ claripennis$ (Cc), † above bar: no significant trend to express preference for one instead of other prey type. All other bars: significantly more test spiders chose sugar-fed Anopheles (P < 0.05). The number of spiders that chose both prey is indicated in bold italics above bars.

pre-trial fasts than was the case for adults. Another striking finding from simultaneous-presentation testing was that, when fast duration was only 1 day, juveniles chose sugar-fed *Anopheles* in preference to blood-fed *Culex*. In this instance, the juvenile's priority appeared to be for *Anopheles* instead of a blood meal. This was a sharp contrast with the findings from adults, where blood meals were the highest priority whenever preferences were expressed.

Yet another preference seems to be superimposed on preference for blood meals and for *Anopheles*. Juveniles and adults of *E. culicivora* expressed a preference for female instead of male mosquitoes independent of acquiring a blood meal, but only if the female mosquito was *Anopheles*. In the absence of blood, there was no evidence that *E. culicivora* discriminated between male and female *Culex*.

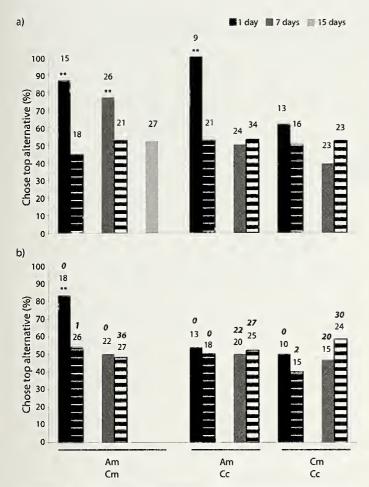


Figure 8.—Expression of preference by juveniles (solid bars) and adults (hatched bars) of *E. culicivora*. Pre-trial fast durations indicated by different shadings. Each individual spider tested with male mosquitoes (*Anopheles* and *Culex*) (percent choice of top alternative prey type depicted in bar) and other prey (both prey types in text underneath line below graphs) (N used for statistics over bar). a) Simultaneous-prey tests (data analysis: test of goodness of fit, $H_o = 50/50$). b) Alternate-day tests (data analysis: McNemar tests for significance of changes). Prey: *Anopheles* male (Am), *Culex* male (Cm), *C. claripennis* (Cc). Results from tests all ns unless stated otherwise above bar (** P < 0.01). In alternate-day tests the number of spiders that chose both prey is indicated in bold italics above bars.

Why the Anopheles-Culex distinction matters to the small juveniles of E. culicivora may be explained by the small size of these spiders. The smallest instars of E. culicivora juveniles are only 1–2 mm in body length and yet these mite-size spiders prey on the much larger mosquitoes. This is not a trivial undertaking because the mosquito sometimes takes flight and shakes the spider off. However, small juveniles adopt Anopheles-specific prey-capture behavior (Nelson et al. 2005) that critically relies on the mosquito's resting posture. Culex rests with its body parallel to the substrate, but Anopheles rests with its body at an angle to the substrate (head down and abdomen elevated: Clements 1999). Larger spiders attack mosquitoes from almost any orientation, but the mite-size juveniles maneuver so that they approach the resting Anopheles from behind, moving slowly under the mosquito's elevated abdomen before leaping up and grabbing hold of the mosquito close to the junction between its thorax and

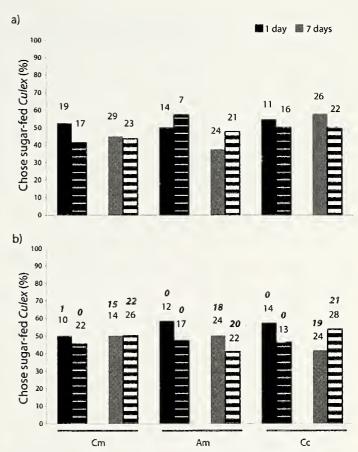


Figure 9.—Expression of preference by juveniles (solid bars) and adults (hatched bars) of E. culicivora. Pre-trial fast durations indicated by different shadings. Each individual spider tested with sugar-fed Culex female and with another prey. Data expressed as percentage of test spiders that chose sugar-fed Culex females (N used for statistics over bar). a) Simultaneous-prey tests (data analysis: test of goodness of fit, $H_o = 50l50$). b) Alternate-day tests (data analysis: McNemar tests for significance of changes). Other prey: Culex male (Cm), Anopheles male (Am), C. claripennis (Cc). All tests ns. In alternate-day tests the number of spiders that chose both prey is indicated in bold italics above bars.

abdomen. The mosquito may take flight, but from underneath the small spider generally maintains its grip and, presumably owing to the spider's venom taking effect, the mosquito usually soon fails to the ground with the predator on board. By expressing preference for *Anopheles*, the small juvenile targets the particular mosquitoes it is most proficient at capturing.

It is harder to explain what larger individuals of *E. culicivora* might gain by expressing a preference for *Anopheles*. Once seized by an adult or a large juvenile of *E. culicivora*, the likelihood of breaking free appears to be close to nil for a mosquito, whether it be *Anopheles* or *Culex*. On the other hand, the expression of a strong preference by juveniles and adults of *E. culicivora* for blood-carrying mosquitoes suggests that there are metabolic benefits from blood meals, this being a hypothesis that we are currently investigating. One nonmetabolic role of blood meals may be that, by feeding on blood-carrying female mosquitoes, adult females and males of *E. culicivora* acquire an odor that renders them more attractive to the opposite sex (Cross et al. 2009), suggesting that

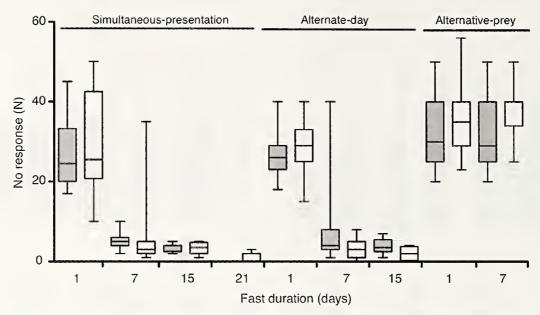


Figure 10.—Boxplots (median and quartiles) with whiskers (min and max) for numbers of juvenile (shaded) and adult *E. culicivora* that did not respond in the three testing protocols for each of the fast durations.

preference for blood meals by adults is driven, at least in part, by sexual selection. We are also investigating whether *Anopheles* is an optimal source of nutrients or of mate-attracting odor, but there is currently no evidence supporting either hypothesis. Alternatively, a preference for *Anopheles*, when expressed by larger individuals of *E. culicivora*, might be explained largely as a trait carried over as a relic from when these individuals were small juveniles.

Our findings are consistent with an important prediction from foraging models (Pyke et al. 1977; Caraco et al. 1980; Stephens & Krebs 1986; M^cNamara & Houston 1990; Toft & Wise 1999), as we have shown that pronounced preference for a specific prey type veers toward indiscriminate response to prey after lengthy fasts. However, whether *E. culicivora* often experiences fasts sufficient to erode preference in the field is debatable. *E. culicivora* is a mosquito specialist, but this does not mean it feeds on mosquitoes alone. *E. culicivora* preys on other arthropods, including midges (chironomids and chaoborids) that are notoriously abundant along the shoreline of Lake Victoria (Beadle 1981).

Preferences, being cognitive attributes inherent to a predator, are knowable only by data from experiments designed specifically for determining preference. Along with araneophagic and myrmecophagic salticids, E. culicivora is a specialized predator that expresses preferences that are applicable to particular kinds of prey-in this case blood meals, Auopheles instead of Culex mosquitoes, and female instead of male *Anopheles*. Our results illustrate that the three testing protocols are useful to identify different levels of preference. Being so stringent, obtaining 'choice' responses during alternative prey testing was very difficult and probably only simultaneous presentation and alternate day tests with a variety of fasting periods are sufficient to extract such detailed information. By adding different hunger levels, we were able to obtain precise information about the different prey choice decisions made by juvenile and adult E. culicivora that would not have been illustrated without this series of different testing protocols and fasting times. In general terms, the easier the test, the longer the fasting time required to 'break' the preference, such that invariably preference was lost in less time for alternative-prey tests than alternate-day tests, and preference in these, in turn, lost its expression before simultaneous-presentation tests. This methodology therefore can be of considerable use to determine preypreference in animals.

ACKNOWLEDGMENTS

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Characterization of the thermal micro-environment of *Paraphysa parvula* Pocock 1903 (Araneae: Theraphosidae), a spider from the Chilean Andes

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Abstract. We characterize, in both the laboratory and the field, the preferential thermal microenvironments of Paraphysa parvula (Pocock 1903) (Araneae: Theraphosidae), a mygalomorph spider that successfully inhabits the high elevation environments of the Chilean Andes. We studied 116 spiders. Their average body temperature in the field was $31.02 \pm 2.74^{\circ}$ C, similar to the laboratory preferred temperature of $31.7 \pm 2.31^{\circ}$ C, and higher than the ideal temperature of reproductive females, $29.34 \pm 2.81^{\circ}$ C. In non-reproductive spiders, we found significant associations between body temperature and the temperatures of the air, substrate and rocks; however, the strongest association was between body and rock temperatures. Similar results were obtained in reproductive females, but there the best predictor of the body temperature was air temperature in the shelter. In both cases, the air temperature remained below body temperature and well below the temperature of the rocks and stones. Both situations show the importance of behavioral thermoregulation and the mechanisms of heat transfer into the microenvironment in the body temperature regulation of spiders. Conduction from the environment, heat transfer by small convection currents, and radiation from the hot stones constitute small environmental cues that allow these spiders to maintain an optimal temperature. The selection of shelters meeting specific temperature regimes appears to be a key condition for the optimization of female reproductive success and survival of females and juveniles in a high elevation environment.

Keywords: Behavioral thermoregulation, mygalomorph spiders

In ectothermic animals, the selection of temperatures can influence many aspects of life history such as aging, habitat selection, mating, and development (Canals 1998; Angilletta et al. 2002). This is especially relevant in spiders, for which thermal limits may be helpful in assessing the suitability of foraging and nesting sites, particularly for species in which the female stays in her nest with her egg sac (Hanna & Cobb 2007). The thermal biology of spiders has been poorly studied and limited to understanding the thermal tolerances of a few species and their relation to the habitat (Humphreys 1987; Schmalhofer 1999; Hanna & Cobb 2007). To the best of our knowledge, with reference to mygalomorph spiders, only the preferential temperature of Aphonopelma sp. has been reported (Seymour & Vinegar 1973; Schmalhofer 1999). Spiders are small, which results in a large body surface area per unit mass, making them susceptible to rapid heat loss, heat gain, and water loss. This is particularly relevant in the conditions of high temperature and low humidity that are typical of xeric environments. As ectotherms, their metabolism is temperature dependent, which means that their oxygen consumption and carbon dioxide production increase, following a power law, with increasing environmental temperature. This could conflict with the conservation of water as a result of high water exchange rates associated with high metabolic rates and increased evaporation at high environmental temperatures. This is especially critical in the mygalomorph spiders that have two pairs of book lungs with a surface area that is also large (Canals et al. 2007). Thus, the involvement of the book lungs in evaporative water loss can reach 50% (Davies & Edney 1952) or 60% (Figueroa et al. 2010). In other spiders, the relationship between temperature and evaporation

is also evident. For example, lycosid spiders of xeric environments have lower evaporation rates than those living in caves (Hadley et al. 1981), and the low evaporation rates of the widow spider Latrodectus hesperus Chamberlin & Ivie 1935 appears to have allowed successful colonization of desert habitats in southwestern North America (Hadley & Quinlan 1989).

The Chilean Andes are characterized by large daily and seasonal temperature variability, which, depending on the substrate, can range from several degrees below 0° C in winter to above 40° C in summer, and large changes in water vapor pressure and availability of prey for spiders (Canals et al. 2007). Under high temperature conditions, mygalomorph spiders maintain a low metabolism compared to other arthropods (Anderson 1970; Greenstone & Bennett 1980; Anderson & Prestwich 1982; Figueroa et al. 2010) that can be supported by a low number of prey. In mygalomorphs, high temperatures can cause metabolic depression (Canals et al. 2007) or elevation of metabolism that can lead to dehydration (Figueroa et al. 2010).

The mygalomorph spider *Paraphysa parvula* (Pocock 1903) successfully inhabits these high elevation environments in the Chilean Andes at altitudes above 2000 m. This species dramatically increases its rate of evaporative water loss by about 10 times when it is moved from 20 to 40 °C, and 40°C appears to be upper temperature limit above which there is danger of dehydration (Figueroa et al. 2010). This spider is also sensitive to elevated carbon dioxide, which promotes opening of the spiracles and evaporative water loss (Davies & Edney 1952; Figueroa et al. 2010). Under the conditions of high summer temperatures of the Andean highlands, these spiders face the possibility of losing water and should seek temperate environments and oxygenated shelter under rocks or bushes.

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Table 1.—Distribution of body mass (mb) of *Paraphysa parvula* spiders captured in the field.

mb (g)	N (%)	
0.0-0.5	64 (55.2)	
0.5-1.0	30 (25.9)	
1.0-2.0	9 (7.8)	
2.0-4.0	5 (4.3)	
4.0-6.0	3 (2.6)	
6.0-8.0	5 (4.3)	

In this article, we studied and characterized the preferential thermal microenvironments of *Paraphysa parvula* in the laboratory and the field. Specifically, we studied the preferred temperature in both situations and analyzed whether the selection of shelters was related to the selection of specific thermal microenvironments.

METHODS

Animal model, capture, and maintenance of individuals.—The animal model was *Paraphysa parvula* (Pocock 1903) (Araneae: Theraphosidae), an inhabitant of the central mountains (elevations above 2000 m) of the Chilean Andes. It is a crepuscular and nocturnal spider, although males of this species occasionally can be seen at noon. During the day, it can be found in shelters under flat stones. Its reproductive period occurs between December and January. The body mass of adults ranges between 6–10 g.

All animals used in this study eame from a population from the central Andes of Chile (Farellones: 33 ° 21 'S 70 ° 20'W) at about 2400 m above sea level. The capture area is dominated by low shrubs, mainly *Chuquiraga oppositofolia*, *Ephedra chilensis*, and *Acaena splendens* (Rosaceae) and numerous small rocks. Once captured, animals were brought to Santiago (550 m) and kept in boxes with natural light and photoperiod.

Field activities.—In the field, we defined 20 quadrats of 5×5 m. In each quadrat, all the stones were lifted and potential shelters were explored. We conducted all observations between 11:00 and 18:00 hours, when the temperature was highest. When a spider was found, its body temperature (Tb) was recorded with a type K thermocouple, which was attached dorsally between the cephalothorax and abdomen. We also recorded air temperature (Ta) in the shade at 1 m height, the temperature of the nearest rock (Tr) and soil temperature (Tt). All animals were weighed (mb) using a digital balance (± 0.01 g). We studied a total of 116 individuals under this protocol.

Subsequently, a second sampling was performed during the reproductive season, exclusively focused on adult females with egg sacs; we studied 15 spiders (mb = 5.33 ± 1.54 g). The procedure was similar to that described above, except that as

we found them under shelters, we noted whether the temperature in the shade corresponded to the temperature of the shelter. When a spider was detected, Tb, Ta, Tr and Tt were recorded.

Laboratory.—Seven adult females and 20 juveniles of various developmental stages were selected for the experimental study of preferred temperature (mb ranged between 0.4-11.4 g). We created a thermal gradient from 10° to 70° C in an opaque plastic cylinder 70 cm long and 5 cm in diameter, with a continuous record of temperature at four equidistant points using K-type thermocouples. The spiders were introduced individually into the gradient and they were neither watched nor perturbed during the trial. They could move freely, selecting their temperature for three hours, enough time to establish a thermal equilibrium with the environment. At the end of the experiment, we removed the subjects and within the first 10 s recorded the body temperature with a type K thermocouple attached dorsally between the cephalothorax and abdomen, using a digital thermometer (EXTECH Instruments, model AE15). That recorded temperature was considered as the behavioral preferred temperature.

Analysis.—We calculated descriptive statistics of body temperature for the three situations: preferred temperature in the laboratory, operating temperature, and field temperature of females of reproductive age. These temperatures were compared with one way ANOVA. We then performed regression analysis between mass and body temperature.

Univariate and multiple regressions of Tb against substrate temperatures (Ta, Tr, and Tt) were performed, the latter with stepwise (backward) selection. We performed correlation analysis between these variables.

RESULTS

We studied 116 spiders with body masses between 0.02-8.0 g (Table 1). The body temperature was $31.02 \pm 2.74^{\circ}$ C (average \pm standard deviation), which was similar to the laboratory preferential temperature, $31.7 \pm 2.31^{\circ}$ C, and higher than the preferential temperature of reproductive females, $29.34 \pm 2.81^{\circ}$ C (F2.99 = 51.65, p = 0.028; Table 2). Correlation between temperature and body mass was not significant in the laboratory (F1,25 = 0.21, p > 0.05), in field non-reproductive spiders (F1,59 = 0.33, p > 0.05), or in reproductive females (F1,12 = 0.60, p > 0.05) (Figs. 1–3).

In non reproductive spiders, significant regressions between Tb and Ta (Slope $\beta=0.69;\ r2=0.33;\ F1,114=58.22,\ p\ll0.001);\ Tb and Tr (<math display="inline">\beta=0.46;\ r2=0.53;\ F1,114=126.59,\ p\ll0.001)$ and Tb and Tt ($\beta=0.32;\ r2=0.40;\ F1,114=77.68,\ p\ll0.001)$ were found (Fig. 4). All these variables were correlated (Table 3), and when a stepwise multiple regression was performed, the selected model was Tb = 11.02 + 0.46 Tr. Similar results were obtained in reproductive females: Tb and

Table 2.—Preferred body temperature in the laboratory and in the field in non-reproductive and reproductive individuals (°C). Different letters indicate significant differences ($\alpha = 0.05$) in multiple comparisons.

	Laboratory	Non reproductive (field)	Reproductive females (field)
Mean ± S.D.	$31.70 \pm 2.31a$	$31.02 \pm 2.74a$	$29.34 \pm 2.81b$
95% confidence interval	30.78-32.61	30.32-31.72	27.71-30.96
Range	26.2–36.8	24.4-37.0	23.1-33.0
Median	31.8	31.4	29.95

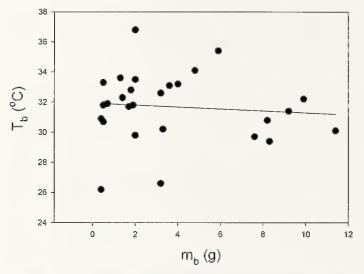


Figure 1.—Variation of the preferred body temperature (Tb) of *P. parvula* as a function of body mass (mb) in the laboratory. The line is the estimated linear regression.

Ta ($\beta=0.53$; r2 = 0.79; F1,12 = 51.30, p < 0.001); Tb and Tr ($\beta=0.21$; r2 = 0.34; F1,12 = 7.81, p < 0.016) and Tb and Tt ($\beta=0.38$; r2 = 0.47; F1,12 = 12.52, p < 0.001) (Fig. 5). All variables were correlated (see Table 2); the multiple regression selected the model Tb = 14.51 + 0.43 Ta. The body temperatures of the non-reproductive and reproductive spiders were lower than Tr and Tt, lower than Ta in non-reproductive spiders, and similar to the Ta of reproductive females in shelters (Figs. 4, 5; Table 4).

DISCUSSION

The preferred temperature of non-reproductive *P. parvula* spiders was about 31° C in the laboratory and in the field. Although the difficulty of extrapolating from the laboratory to the field has been noted (Humphreys 1977), our results were very consistent; the mean, median and range were fully matched (see Table 2). The means and ranges were similar to

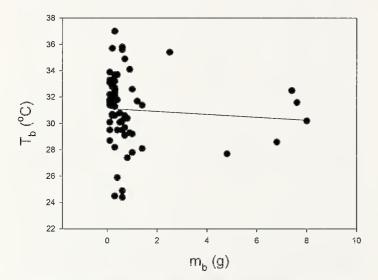


Figure 2.—Variation in preferred body temperature (Tb) in non-reproductive individuals of P. parvula as a function of body mass (mb) in the field. The line is the estimated linear regression.

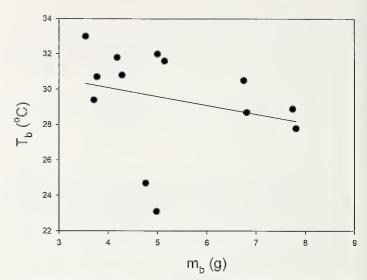


Figure 3.—Variation of the preferred body temperature (Tb) in reproductive females of *P. parvula* as a function of body mass (mb) in the field. The line is the estimated linear regression.

the preferred temperature reported for the American tarantula *Aphonopelma* sp.; shelter temperatures between 27 and 35° C were reported for this species (Seymour & Vinegar 1973). Although there was great variability in body temperature in the juveniles (with low body mass), we found no significant effect of mass on the temperature preferences. This result contrasts with that reported for lycosid spiders (Sevacherian & Lowrie 1972; Humphreys 1975; 1978; De Vito & Formanowicz 2003). For example, Sevacherian & Lowrie (1972) found that juveniles of two *Pardosa* species showed optimal temperatures lower than adults, and De Vito & Formanowicz (2003) found that juvenile riparian spiders (*Pirata sedentarius* Montgomery 1904) exposed to thermal stress survived better than adults.

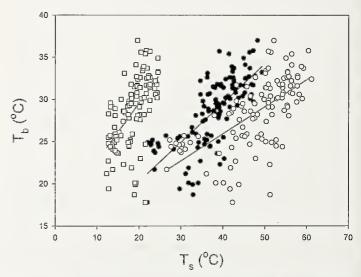


Figure 4.—Variation in body temperature (Tb) in non-reproductive individuals of *P. parvula* as a function of the substrate temperatures (Ts) in the field. White squares indicate air temperature (Ta), black circles indicate rock temperature (Tr) and white circles indicate soil temperature (Tt). The lines indicate the estimated linear regressions.

Table 3.—Correlations between body temperature (Tb) and the temperature of the air (Ta), rocks (Tr) and ground (Tt) for non-reproductive individuals (bold, above diagonal) and reproductive females (below diagonal).

	Tb	Ta	Tr	Tt
Tb		0.58	0.73	0.64
Ta	0.90		0.6	0.67
Tr	0.63	0.48		0.82
Tt	0.71	0.55	0.79	

The upper limit of preferred temperature was about 37° C, lower than the temperature at which water loss increases sharply in this species (Figueroa et al. 2010), and as expected, lower than the maximum critical temperature (maximal temperature at which an animal can display coordinated locomotory behavior) reported for Aphonopelina sp. (43° C). Compared to other species of labidognathan spiders, preferred temperatures of P. parvula are in the upper range, similar to some lycosids such as *Pardosa pullata* (Clerck 1757) (Pulz 1987) and Pardosa sierra Banks 1898 which, like our studied species, are diurnal and nocturnal hunters from temperate zones (Sevacherian & Lowrie 1972). The preferred temperature of females carrying an egg sac was about 3°C lower than the temperature of choice for non-reproductive animals, as was the lower limit of the preferred range of temperatures, while the upper limit decreased 1-2 °C. Higher optimal temperatures have been reported for lycosid females carrying egg sacs (Norgaards 1951; Sevacherian & Lowrie 1972), lower (Humphreys 1978) or equal (Norgaard 1951; Vlijmen et al. 1963; Frick et al. 2007) to those of non-reproductive individuals. High preferential temperatures could accelerate the development of the offspring; however, it has also been reported that high temperatures lead to retarded development in lycosids (Li & Jackson 1996). In our species the preferential temperature of non-reproductive individuals is high, and the choice of a higher

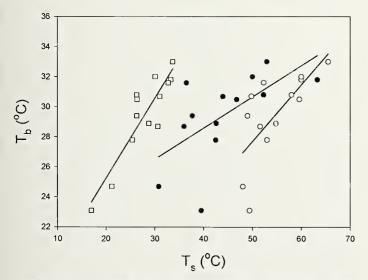


Figure 5.—Variation in body temperature (Tb) in reproductive females of *P. parvula* as a function of the substrate temperatures (Ts) in the field. White squares indicate temperature of the refuge (Ta), black circles indicate rock temperature (Tr) and white circles indicate soil temperature (Tt). The lines indicate the estimated linear regressions.

Table 4.—Body temperature (Tb) of non-reproductive individuals and reproductive females of the spider P. parvula, and the temperature of the air (Ta), rocks (Tr) and ground (Tt). Results are shown as mean \pm standard deviation.

A 1000 -	Non-reproductive individuals	Reproductive females
Tb (°C)	31.02 ± 2.34	29.84 ± 2.81
Ta (°C)	18.46 ± 3.49	27.97 ± 4.65
Tr (°C)	37.66 ± 6.46	43.98 ± 8.15
Tt (°C)	48.03 ± 8.22	55.13 ± 5.26

temperature by reproductive females could have an effect on water loss or produce a metabolic depression in the offspring.

Both in non-reproductive animals and in the reproductive females, the body temperature change per degree C of temperature change in the physical environment (Ta, Tr and Tt) was less than 1° C, which was associated with slopes lower than 1 in all simple regressions. This indicates that P. parvula thermoregulated behaviorally, seeking shelters that allowed them to maintain temperatures varying less than in the environment. Ambient temperature (Ta), that of the nearest rock (Tr) and soil temperature (Tt) were strongly correlated with the body temperature of spiders, but while the temperature of soil and rock varied between 30–60° C, body temperature of spiders remained between 27.71–32.61° C. This is similar to results reported for Aphonopelma sp., in which the temperature of the soil adjacent to the entrances of their eaves reached 55° C, while the temperature of the shelter was only 36° C, maintaining the area adjacent to the spider usually between 27.2-30.0° C (Seymour & Vinegar 1973). The air temperature at one meter in the shade ranged from 15–25° C for non-reproductive spiders, while air temperature in the shelters of reproductive females varied between 18–32° C. In both cases, the air temperature remained below body temperature and well below the temperature of the rocks and

In the non-reproductive animals, the model that best explained an animal's body temperature was the temperature of the surrounding rocks. For the females in their shelters under rocks, it was not the surface temperature of the stones that best explained the body temperature, but rather the air temperature in the shelter. Both situations show the importance of mechanisms of heat transfer into the microenvironment on the body temperature regulation of spiders. The processes of conduction from the environment, heat transfer by small convection currents, and radiation from the hot stones constitute small environmental cues that allow these spiders to maintain an optimal temperature. In this context, the selection of shelters that meet specific temperature conditions appears to be a key condition for the optimization of female reproductive success and survival of females and juveniles in a high altitude environment.

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Spider guilds in the tree-shrub strata of riparian forests in southern Brazil

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Abstract. We evaluated spider guild abundance and vegetation complexity in riparian forests in southern Brazil in four distinct river basins over 2 yr. We compared spider guild abundance among rivers, habitats (edges vs. forest interior), and against vegetation complexity. We also compared spider assemblages between juvenile and adults in terms of guilds. Of 42,057 spiders sampled, 79% were juveniles and 21% were adults. Space-web weaving was the most abundant guild whereas cursorial hunters was the least abundant one. Weaving spiders dominated over hunters (59% vs 40.58%, respectively). Thirty-four families were recorded; ambush hunters totaled 11 families; space web sheet builders and hunting runners had eight families each and there were seven families for orbicular web builders. Space web sheet builders dominated on all levels: among rivers and habitats (edges and forest interior). Unexpectedly, spider guilds did not seem to be influenced by environmental complexity, given the variables measured, under a Canonical Correspondence Analysis. There was an interaction between guild relative abundance and ontogeny, since the proportion of the space web sheet builders guild among adult spiders was larger than the proportion among juveniles, with a decrease in proportion of adults especially for hunting runners.

Keywords: Guild composition, diversity, beating tray, habitats, vegetation complexity

Spider guilds are usually classified based on hunting strategies and predation habits (Uetz 1991; Silva 1996; Uetz et al. 1999; Höfer & Brescovit 2001). According to Simberloff & Dayan (1991), a guild is defined as a group of species exploiting the same environmental resource classes in a similar fashion.

In Brazil, Höfer & Brescovit (2001) analyzed spiders and their guilds in Ducke Reserve, Manaus, in the Brazilian Amazon, aeross several years. Given the high diversity of tropical spider assemblages and the lack of knowledge on species' biology, they recognized future guild classifications can potentially be distinct from the present ones.

Other studies in Brazil (Battirola et al. 2004; Oliveira-Alves et al. 2005; Peres et al. 2007; Souza-Alves et al. 2007; Rodrigues et al. 2009; Dias et al. 2010), South America (Silva 1996; Silva & Coddington 1996; Flórez 1999; Avalos et al. 2007; Benavides & Flórez 2007; Rubio et al. 2008) and elsewhere in the world (Jennings & Hilburn 1988; Uetz et al. 1999; Toti et al. 2000; Whitmore et al. 2002; Chen & Tso 2004; Sørensen 2004; Laeser et al. 2005; Loeser et al. 2006; Hore & Uniyal 2008), focused in various aspects of spider guilds, especially their differential occurrence in distinct environments, plant strata and capturability by different collection methods.

Even though spiders are recognized as being very important ecologically (Simó et al. 1994), especially as essential components of forest ecosystems (Moulder & Reichle 1972), studies exploring this taxon as an indicator of habitat disturbance (Cardoso et al. 2010) and the effects of its predatory function in the ecosystem are still incipient for many regions, markedly tropical and subtropical ones. Although the literature on spider guilds has focused in habitat use and occupancy, many environments and vegetation physiognomies

are still understudied, and among those stand the riparian forests

Riparian forests are those kinds of vegetation associated to water courses (Ab'Saber 2000). In Brazil they are legally protected (Brazilian Forest Code, law 477/1965) and considered permanent preservation areas, but that has not prevented them from being degraded. This is clearly a case of bad management of natural resources (Kilca 2002), with important societal ramifications ranging from lower food production, lost opportunities for ecotourism and unknown levels of biodiversity loss (Malavasi et al. 2004).

Several studies demonstrate that changes in vegetation surrounding rivers, streams and brooks affect the associated invertebrate fauna (Nakano & Murakami 2001; Kato et al. 2003; Baxter et al. 2005). The resident fauna of riparian forest edges is also affected (Laeser et al. 2005). Riparian forests can work as corridors for spider dispersal between distant ecosystems (Raizer et al. 2005). However, human disturbance on these forests can affect this flow or even halt it. Hence, there is an urgent need to know and characterize the spider fauna associated with riparian forests in Brazil. The aim of this research was to compare the abundance of spiders guilds oecurring in different riparian forests (drainage basins), among forest habitats (edges and forest interior), and differences found among guilds for adult and juvenile spiders. Distinct methods for measuring forest structure and complexity and how the latter influence spider guilds were also evaluated.

METHODS

Study areas.—Samples were taken from riparian forests in four different drainage basins in southern Brazil, state of Rio Grande do Sul (Fig. 1).



Figure 1.—Schematic map of Brazil and Rio Grande do Sul State. State map shows the studies riparian forests. See Methods for abbreviations.

- 1. Piratini river (PR): Sampling area is on the North bank of the lower Piratini, in the southern region of the Coastal Plain in Arroio Grande municipality (31°54′06.47″S, 52°39′08.29″W). The selected forest section is approximately 14 m a.s.l. and 39 km long down the valley, having 4,000 ha of riparian forest, representing the largest native continuous forest in this region currently. Temperatures are on average 18.2° C with February the hottest (23.4° C) and July the coldest month (10.2° C); annual rainfall reaches 1,283 mm, with August the rainiest month (123 mm) and January the drier (48 mm) (Oliveira & Ribeiro 1986). According to Teixeira et al. (1986) the Piratini river vegetation on its lower course has areas with Pioneer Formations with riverine and marine influences. Typical plant species include Allophylus edulis (Sapindaceae), Engenia uruguayensis (Myrtaceae), Trichilia claussenii (Meliaceae), Banara tomentosa (Salicaceae), Gymnanthes concolor (Euphorbiaceae) and Chrysophyllum marginatum (Sapotaceae) (Kilca 2002).
- 2. Camaquã river (CR): Study area is on the North bank of the lower Camaquã (31°01'01.7"S, 51°56'42.0"W), on the centresouth portion of the Coastal Plain in Cristal municipality. It is

also approximately 14 m a.s.l. and suffers frequent seasonal flooding. The area is in a good conservation state, with forest continuity over an alluvial plain and low indication of human disturbance (Marchi 2005), although the forest is not particularly tall. Average annual temperatures are 18.9° C, with July colder (13.3° C) and January/February warmer (24.3° C). Average annual rainfall is 1,234 mm, with September the rainiest month (135 mm) and November the driest (65 mm) (IPAGRO 1989). Marchi (2005) mentions the largest trees in the region being Luehea divaricata (Malvaceae), Salix humboldtiana (Salicaceae) and Vitex megapotamica (Lamiaceae), also with shrub components Psychotria carthagenensis (Rubiaceae) and Ruellia angustiflora (Acanthaceae). Amongst the most abundant trees in Camaquã river there are Sebastiana commersoniana (Euphorbiaceae), Engenia verticillata (Myrtaeeae), Allophylus edulis, Cupania vernalis (Sapindaceae) and Gymnanthes concolor.

3. Sinos river (SR): The study area is in Parobé, South bank of the river (29°41′06.94″S, 50°51′05.98″W), altitudes between 6–10 m a.s.l. (Daniel 1991). The forest is not continuous, with fragmentation along the river, but the chosen sampling spot includes the largest patch of forest in the region. On the North

bank there is a beach used by locals (Daniel 1991), but on the South bank the forest is taller, surrounded by flooding grasslands, wetlands and, further on, enclosed pastures. The highest monthly average temperatures reach 22° C and the coldest are 3–18° C. Sinos river rainfall is 1,200–1,750 mm annually (Daniel 1991; Diesel 1991), with monthly averages between 90 (drier) and 190 mm (rainiest), and rains more common in winter months (Oliveira & Ribeiro 1986), leading to higher river levels. In the study area large deciduous trees like *Luehea divaricata* and *Anadenanthera macrocarpa* (Fabaceae) can be found, in winter leaf loss occurs quickly revealing the semideciduous character of such forests (Daniel 1991). Shrubs are uniformly distributed in the area, especially *Psychotria leiocarpa*, *P. myriantha* (both Rubiaceae), and *Justicia brasiliana* (Acanthaceae) (Diesel 1991).

4. Maquiné river (MR): This study area is in a forest fragment on the East bank of Maquiné river (29°40'47.99"S, 50°11'20.03"W), of which the whole valley covers 622 km², within the Serra Geral slopes region adjacent to the Coastal Plain (Sevegnani & Baptista 1995). This region is part of the UNESCO Atlantic Forest Biosphere Reserve since 1992. Within Maquiné's drainage basin there is a conservation unit (Reserva Biológica da Serra Geral), comprising 4,845 ha. The region is considered of extreme importance to the conservation of the Brazilian Atlantic forest (Conservation International do Brasil et al. 2000), for its high biodiversity but also fragility to anthropization. Average temperatures for the coldest month are 13-15° C and for the warmest month are 23-25° C; annual rainfall ranges from 1,400 to 1,800 mm, with elevated frequency of rainy days throughout the year (Oliveira & Ribeiro 1986). Natural vegetation in the valley, although mainly represented by dense humid forest, is a biogeographic transition with elements of the semideciduous seasonal forest. Land cover is extremely heterogeneous, a mosaic of primary and secondary vegetation at various stages of development along with agriculture areas (Sevegnani & Baptista 1995).

Sampling.—Sampling occurred across 2 yr (01 August 2007-06 June 2009), with two samples per season on each of the four regions studied. In the subtropical region this study was undertaken, there are four distinct seasons in terms of temperature, with rainfall evenly distributed or with a slight predominance of rains during winter. In each riparian forest, parallel transects were established in three habitats within the forest: the closest possible to the edge of the forest with the river; the closest possible to the edge of the forest with the adjoining grassland/pasture and the forest interior as far as possible from either edge. These three transects constituted a set; the minimum distance between transects within a set was 20 m. Two sets of transects were established per riparian forest. Each transect was a straight line approximately 50 m long; sampling never exceeded 2 m on each side of this line. Overall, 24 transects were sampled per sampling date among all areas.

Spiders in the tree-shrub layer were sampled with a beating tray during 45 min on each transect, totaling 288 h of sampling. Beating was employed on vegetation between 0.5 and 1.5 m. This method is efficient in sampling spiders living on small and medium sized shrubs, tall herbs, woody lianas, small trees and shoots of larger trees (Coddington et al. 1996; Sørensen et al. 2002). The beating tray was a wood structure

mounted as a cross (70×70 cm) covered by a white nylon sheet. Spiders were transferred to 80% ethanol on the spot.

Spiders were deposited in the Museu de Ciências Naturais of Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil (MCN/FZB, curator: Erica H. Buckup).

Environmental complexity.—Vegetation structure was evaluated with four different methods. For vegetation density four transects were established in the same areas where spiders were sampled. Each transect was determined as a 25 m long line held 1 m above the ground, from which vegetation touches to the line were counted. At the starting point of each transect the vegetation vertical structure was evaluated, with a 2 m long, 20 mm diameter rod. The rod was held upright and the number of vegetation touches to the rod was counted. At this same point, vegetation height was measured to an approximation with the help of a 3 m long rod hold high by one of the sampling crew, observed by another sampler from a fixed (10 m) distance. Vegetation cover was sampled at the same point in the transect from a printed (20 × 20 cm) photograph taken up at a fixed height (1 m); a transparent grid (10 × 10 mm) was placed on the photograph and the number of squares with more than 50% of its area covered was counted. All measures are modified from Raizer (2004) and Raizer et al.

Guild membership.—As a basis for guild separation we used Silva (1996), Uetz et al. (1999), Höfer & Brescovit (2001) and Rodrigues et al. (2009). All spiders, separated by families, were grouped in one of the following guilds: a) web spinners: 1. orb weavers (construct bidimensional webs) and 2. space web sheet builders (construct tridimensional webs); b) hunters: 1. hunting runners (search and hunt their prey actively) and 2. ambushers/stalkers (do not build webs but sit-and-wait for their prey).

Data analysis.—Three factors of interest were tested: river basins (four levels: Piratini, Camaquã, Sinos and Maquiné); habitats (three levels: river edge, forest interior and grassland edge); and spider maturity (two levels: juvenile and adult). A MANOVA (multivariate analysis of variance) was employed to compare guild abundance proportions (arcsine transformed) with factor levels, implemented in PASW (SPSS®) 18.0. To test for vegetation structure effects and habitats on the relative proportion of spider guilds a MANCOVA (multivariate analysis of covariance) was employed with habitat as the single factor and four quantitative vegetation variables (vegetation density, structure, height and cover) as covariates. In both cases Pillai's Trace was the chosen statistic. To illustrate the effect of vegetation structure and habitats on proportional abundance of the spider guilds, an ordination was developed (Canonical Correspondence Analysis, CCA, Ter Braak 1986). The CCA was implemented in PASt (Paleontological Statisties 1.87b) (Hammer and Harper 2008).

RESULTS

Overall 42,057 spiders were obtained, juveniles more abundant than adults (79% and 21%, respectively); family richness reached 34 (Table 1). Web builders were prevalent (n = 24,992; 59.4%) over hunters (17,065; 40.6%). The most abundant guild was space web sheet builders (n = 16,308 individuals, eight families) (Fig. 2) dominated by Theridiidae (77.4%) and Linyphiidae (19%). The least abundant guild was

Table 1.—Spider guilds (juvenile and adults) and respective families in different riparian forests of southern Rio Grande do Sul, Brazil.

		Riparian	forests			2000 may 2722
Guilds/Families	Piratini	Camaquã	Sinos	Maquiné	Total	%
Orb weavers						
Araneidae	1,900	1,357	749	1,016	5,022	11.941
Tetragnathidae	399	385	246	280	1,310	3.115
Theridiosomatidae	421	357	203	203	1,184	2.815
Uloboridae	237	168	391	208	1,004	2.387
Nephilidae	21	22	43	5	91	0.216
Deinopidae	10	19	15	17	61	0.145
Mysmenidae	3	7	2	-	12	0.029
Space web sheet builders						
Theridiidae	4,356	3,423	2,348	2,491	12,618	30.002
Linyphiidae	284	258	300	2,253	3,095	7.359
Pholcidae	1	115	98	163	377	0.896
Hahniidae	-	-	1	95	96	0.228
Scytodidae	-	11	3	58	72	0.171
Amaurobiidae	6	5	2	9	22	0.052
Dictynidae	1	-	14	6	21	0.050
Synotaxidae	-	-	-	7	7	0.017
Ambushers/stalkers						
Thomisidae	1,242	1,439	823	697	4,201	9.989
Salticidae	719	1,042	1,129	983	3,873	9.209
Mimetidae	115	328	145	63	651	1.548
Pisauridae	46	85	127	271	529	1.258
Sparassidae	-	85	103	73	261	0.621
Philodromidae	1	3	77	79	160	0.380
Senoculidae	51	15	23	38	127	0.302
Oxyopidae	6	11	72	2	91	0.216
Ctenidae	-	-	-	17	17	0.040
Hersiliidae	-		1	-	1	0.002
Idiopidae	-	-	-	1	1	0.002
Hunting runners						
Anyphaenidae	1,933	1,954	1,701	1,210	6,798	16.164
Corinnidae	87	26	16	37	166	0.395
Oonopidae	22	25	6	32	85	0.202
Miturgidae	26	9	11	28	74	0.176
Lycosidae	4	1	8	3	16	0.038
Gnaphosidae	-	3	4	3	10	0.024
Clubionidae	-	-	-	3	3	0.007
Segestriidae	-	-	-	1	1	0.002
Total	11,891	11,153	8,661	10,352	42,057	100
Families	24	26	29	32	34	
Exclusive Families	0	0	1	5		

hunting runners (7,153 individuals, eight families), dominated by Anyphaenidae (95%) and Corinnidae (2.3%). For ambushers there were 9,912 individuals in 11 families with Thomisidae (42.4%) and Salticidae (39.1%) most abundant. Among orb weavers there were 8,684 individuals in seven families, being Araneidae (57.8%) and Tetragnathidae (15.1%) the most abundant families.

Spider guilds and rivers.—In all river sites more web building spiders were found than hunters. There were significant differences in guild proportion among rivers (MANOVA: orb web, F = 17.37, P < 0.001; space web, F = 39.65, P < 0.001; hunting runners, F = 8.59, P < 0.001; ambushers, F = 8.59, P < 0.001; ambushers, F = 8.59, P < 0.001; ambushers, P = 8.59, P <

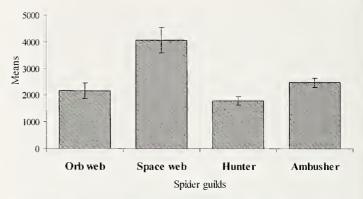


Figure 2.—Spider abundance (average over four rivers ± SE) among guilds in riparian forests of Rio Grande do Sul, Brazil.

30.26, P < 0.001). The most prevalent guild was space web sheet builders (Piratini = 4,648; Camaquã = 3,813; Sinos = 2,767; Maquiné = 5,082), followed by ambushers (Piratini = 2,180; Camaquã = 3,008; Sinos = 2,500; Maquiné = 2,224) on all rivers (Fig. 3) but Piratini, having orb weavers as the second most abundant guild (n = 2,991); on all rivers the least abundant guild was hunting runners (Piratini = 2,072; Camaquã = 2,018; Sinos = 1,746; Maquiné = 1,317) (Fig. 3).

The Synotaxidae family (spacers web sheet build) was exclusive to Maquiné river, as were Clubionidae and Segestriidae (hunting runners), Ctenidae and Idiopidae (ambushers), the latter being the sole Mygalomorphae family recorded here (Table 1). Sinos river had a single exclusive family (Hersiliidae-ambushers).

Spider guilds and forest habitats.—A larger spider abundance was found for the forest interior (n = 16,281; 38.7%), then for the grassland edge (13,121; 31.2%) and the smaller abundance occurred in the river edge (12,655; 30.1%). Guild proportions differed significantly among all habitats (MANOVA: orb web, F = 28.39, P < 0.001; space web, F = 6.79, P < 0.005; hunting runners, F = 7.51, P < 0.003; ambushers, F = 36.61, P < 0.001). The dominant guild is still space web sheet builders; the least abundant guild was hunting runners in the river edge and forest interior, and orb weavers for the grassland edge (Fig. 4). Hunters occurred more on edges; hunting runners in the grassland edge (n = 2,627), ambushers on the river edge (n = 3,605). Weaving spiders were more abundant in the forest interior, for both space web sheet builders (n = 6,943) and orb weavers (n = 4,013) (Fig. 4).

Family richness resulted in 30 recorded in the river edge (one exclusive family – Idiopidae: ambushers), 32 in the forest interior (one exclusive – Segestriidae: hunting runners) and 30 in the grassland edge (one exclusive – Hersiliidae: ambushers).

Spider guilds and vegetation structure.—CCA results are shown on Figure 5. The eingenvalues for the two first axes were: 0.049 (axis 1) and 0.010 (axis 2). The permutation test did not return significant values for axes 1 and 2 (P = 0.42 and 0.24, respectively), indicating the guilds are not significantly correlated to the environmental complexity variables evaluated. The triplot (Fig. 5) demonstrates the first axis to be related to vegetation structure variables (positively) and to vegetation density (negatively). The second axis appears positively related to cover and height. Habitats occur around the origin, being indifferent to the evaluated variables, except forest interior in Maquiné, tending to

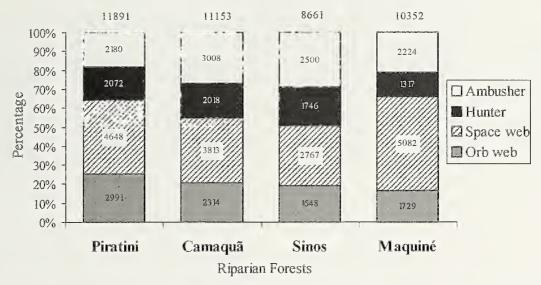


Figure 3.—Spider abundance (juvenile and adults) among guilds in riparian forests of southern Brazil.

remain close to vegetation density (densveg) and forest interior in Piratini, close to vegetation structure (struveg). The MANCOVA did not reveal any significant differences in guild proportions for any of the environmental complexity variables, except space web sheet builders having a significant influence of vegetation cover (F=8.17; p=0.03) and vegetation density (F=7.03; p=0.04).

Ontogenetical changes in spider assemblages.—Comparing adult and juvenile spider assemblages, strong shift can be found regarding guild abundance. Among the juveniles, 47% are hunters and 53% weavers; among adults, only 17% are hunters and 83% weavers.

There are large statistical differences among guild proportions contrasting adult and juvenile spiders (MANOVA: orb weavers, F=173.67, P<0.0001; space web sheet builders, F=1837.56, P<0.0001; hunting runners, F=879.60, P<0.0001; ambushers, F=375.34, P<0.0001). Juvenile spiders have more homogeneous abundances among guilds, whilst adults have a large difference between space web sheet builders and

the other guilds. There is an especially large decrease in the proportion of hunting runners in the passage to the adult stage (Figs. 6, 7).

Among different rivers, juvenile spiders (Fig. 6a) show consistent abundance patterns among guilds, with higher abundances for space web sheet builders in riparian forests of Piratini and Maquiné rivers and more ambushers in Camaquã and Sinos rivers. In terms of adults (Fig. 6b), there is a decrease, especially in hunting runners, and a strong increase in the proportion of space web sheet builders for all rivers (significant interaction: orb web, F = 6.74, P < 0.002; space web, F = 14.85, P < 0.0001; hunting runners, F = 5.45, P < 0.005; ambushers, F = 11.01, P < 0.0001).

There is an increase in the proportion of space web sheet builders for adults also for habitats (Fig. 7) (MANOVA: space web, F = 17.49, P = 0.0002) with the other guilds also differing significantly among habitats, except orb weavers (F = 1.37, P = 0.27). For hunting runners, juvenile cursorials (Fig. 7a) have a higher proportion compared to adults (Fig. 7b).

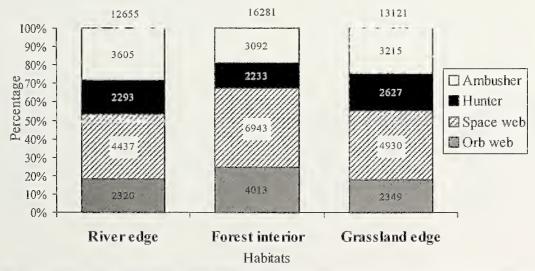


Figure 4.—Spider guild abundance (juvenile and adults) in the three habitats of riparian forests of southern.

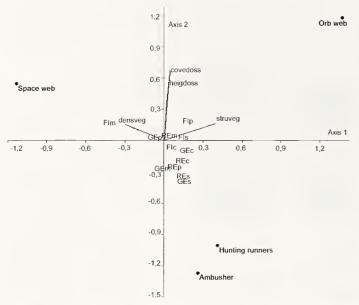


Figure 5.—Canonical correspondence analysis for spider guild abundance (juvenile and adults) response to environmental complexity variables evaluated among habitats in riparian forests in southern Brazil (RE: river edge, FI: forest interior, GE: grassland edge; p: Piratini river; e: Camaquã river; s: Sinos river; m: Maquiné river; covedoss: canopy eover; heigdoss: canopy height; densveg: vegetation density; struveg: vegetation structure).

DISCUSSION

The variation in guild proportions for the different rivers evaluated demonstrates that vegetation physiognomy influences the functional relationship between spiders and their environment. However, there is a degree of homogeneity among rivers in spider guild proportions that suggests a model for the tree-shrub strata of riparian forests of south Brazil: a large abundance of spiders in the space web sheet builders guild and a lower representation for hunting runners. This distinguishes the tree-shrub strata fauna functionally from other habitats occupied by spiders within a forest, such as the underground or topsoil, where hunting spiders usually dominate (Höfer 1997; Loeser et al. 2006; Peres et al. 2007).

This model has been reported from other parts of the world. Sorensen (2004) recorded a higher abundance and richness for space web sheet builders and lower values for ambushers in Africa. Chen & Tso (2004), in China, equally point out space web sheet builders as the most numerous guild. Also for Flórez (1999), in Colombia, there was a predominance of space web sheet builders followed by hunting runners.

The most abundant guilds in this environment are likely those with families and species able to adequately occupy niches, given structural and spatial characteristics of the environment. The abundance of thin and ramified branches in shrubs and small trees generating a complex three-dimensional spatial structure in riparian forests could possibly explain this occupation of space web spiders in this strata. A lack of preferred hunting and sit-and-wait points within the forest, such as flowers or open spots, could explain the lower numbers of ambushers.

The Theridiidae family has more than 30% of all spiders found in this study, almost double the abundance of the second most common family. It is the prevalent family for various inventories in South America (Silva 1996; Silva & Coddington 1996; Flórez 1999; Benavides & Flórez 2007; Avalos et al. 2007). Theridiidae, along with Linyphiidae, were responsible for the space web sheet builders guild being dominant in riparian forests. The higher abundance of space web, overall, suffers a strong influence of the high numbers of individuals in Maquiné river, and a lower influence from Sinos river. Linyphiidae really determined the higher space web sheet builders abundance in Maquiné river (73% of the spiders were in this family).

In the Samiria river riparian forest of Peru, Silva (1996) recorded a higher abundance of hunting runners and a lower number of ambushers. As they sampled different vegetation strata, guild composition was distinct from what we found, indicating a variability in the fauna probably according to vegetation height. In Colombia, Benavides and Flórez (2007), sampled the Igapó forest influenced by the Taraira river, where cursorials dominated, whilst in the *terra firme* forest, irregular web builders dominated. The seasonal inundation regime is thus a factor that seems to affect the araneofauna composition in Igapó forests. We recorded higher abundances for hunters on forest edges; the river edge had more spiders among ambushers. This environment is usually subject to strong winds and can be influenced by floods, which could have lead to lower abundances for the other guilds.

Orb weavers and space web sheet builders were more abundant in the forest interior, perhaps influenced by abiotic

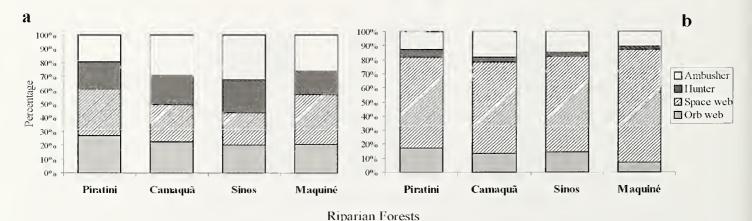


Figure 6.—Spider guilds in riparian forests of southern Brazil. (a) Juvenile. (b) Adults.

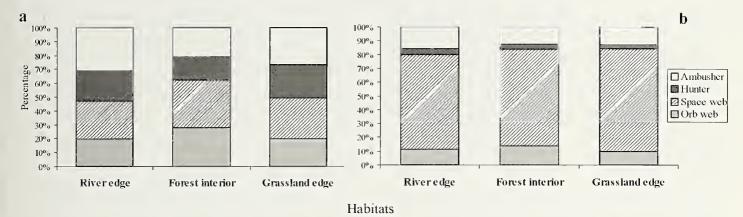


Figure 7.—Spider guilds and habitats in riparian forests of southern Brazil. (a) Juvenile. (b) Adults.

factors on the edges, as floods at river edges, as above, or light and wind intensity at the grassland edge. Among hunters, abundances were homogeneous among habitats, with more individuals on the edges, perhaps related to a hunting strategy independent of web type. According to Baldissera et al. (2004), web distribution and occurrence can be influenced by a forest/edge gradient, because changes in microclimate created by the border can cause thermal tension and damage webs due to the winds.

The forest border is a place where species from different environments can establish and then disperse to the interior of the fragment, creating a flow of immigrants faced by species inhabiting the forest interior. Oliveira-Alves et al. (2005) suggest that the edge effect acts like a natural barrier, impeding the flow of spiders among the environments; these authors found a higher richness for the edge compared to the forest interior.

The lower abundance of orbicular web spinners on edges can be a consequence of the lack of proper habitat and/or wind exposure, being more open, compared to the riparian forest interior, a pattern similar to what is found by Hore & Uniyal (2008). Souza-Alves et al. (2007) recorded more weavers in the forest and more hunters in the grassland; Oliveira-Alves et al. (2005) observed that the hunter guild dominates on the edge and web builders in the forest centre; similar to what was found here. Rubio et al. (2008) observed web builders to be connected to more heterogeneous environments and those with increased structural complexity. Goetze et al. (2001) eoncluded that some spider families have species occurring specifically in some habitats and not in others.

Studies on spider assemblages suggest that faunal composition tends to be strongly related to environmental spatial heterogeneity as determined by the plant community on which spiders live (Gunnarsson 1990; Uetz 1991; Rypstra 1986). Possibly, the occurrence of plants from neighboring environments, such as grasslands, introduces distinct habitats to riparian forests edges. These habitats can be occupied differentially by the spider guilds, some species perhaps coming from the grasslands as well, mixing up the spider fauna of the riparian forest and generating an edge effect for guild composition. Toti et al. (2000) cite differences in physiognomy and plant composition as responsible for differences in guild composition in spider assemblages.

Whitmore et al. (2002) suggest that similar kinds of habitats are similar in their familial spider composition and that there is an influence of habitat structural complexity on guild composition.

Despite considering a suite of factors that can determine riparian forest structure (Ab'Saber 2000), we have failed to find those structural factors that affect spider guilds. Although there are clear effects of habitat on the spider guilds, these guilds were not influenced by the differences found in plant structure and environmental complexity of riparian forests. There are at least two possibilities to explain this: a question of scale and of variation among sampling units. First, the scale at which the environment was evaluated might not be relevant for spiders. The methods are employed and planned by humans and adequate for human perception of the environment, at the scale of meters. Spiders might respond at a smaller scale, that of centimeters of even millimeters. Secondly, riparian forests may present low variation in vegetation structure so as to not affect spider guilds – for spiders with different hunting strategies, such forests are effectively homogeneous. Structural variation must thus be very large to lead to significant changes in abundances, reflecting the opportunistic and generalist character of spiders ecologically (Nentwig 1986; Uetz 1991). Abundance changes in spider guilds of the tree-shrub strata can be more strongly associated to abiotic changes mentioned above, such as wind and light intensity, among other aspects not evaluated here.

Juvenile and adult spider assemblage composition ean be more plastic, changing in response to various factors, such as differences in phenology (maturation time) and differential mortality, among others (Sackett et al. 2008). Frequently, juvenile spiders are not used in quantitative analyses, being discarded and with them a series of valuable information: here we identified a large difference in the pattern of guild abundance between adults and juvenile, that is, the maturation process has distinct consequences for each different spider guild.

Many papers cite hunters (either hunting runners or ambushers) as more abundant on the soil surface (Peres et al. 2007; Loeser et al. 2006) or in the canopy (Silva 1996; Battirola et al. 2004). Probably, the species in these guilds develop such preferences upon reaching the adult stage, being infrequent in the tree-shrub stratum then. Thus, the change in this guild proportions with age could be the consequence of this active vertical selectivity on the part of the spiders.

In our riparian forests, there are families among the hunters recorded only as juvenile. For ambushers: Pisauridae, Ctenidae, Idiopidae and Hersiliidae; for hunting runners: Lycosidae, Gnaphosidae and Segestriidae. All such families except Pisauridae are rare, recorded as a few individuals (< 20 spiders). Possibly, low abundance families are actually spiders not frequently seen in the tree-shrub stratum and could represent "tourists" (Coddington et al. 1996), present accidentally or *en marche* from one environment (soil) to another (canopy). Höfer (1997) mentions that in Amazonia, many spiders migrate vertically during flood seasons, leaving the soil they live on.

A better knowledge of spider guilds, their composition and effect on food webs, among others, are essential in future studies. More abundant guilds can have a fundamental role in the environment, resulting in functional information similar to that of a taxonomic bioindicator. Nowadays, given the scarce knowledge of life history and behavior of most Neotropical species (Höfer & Brescovit 2001; Dias et al. 2010), "higher taxa", such as families for spiders, are increasingly being used in recent studies. According to Uetz et al. (1999) the ideal situation so as to reflect the reality of spider guilds, would be to know the natural history of each species, however this is still far off for most families. Additionally, different classifications are used for the same taxa and groups depending on the authors, which makes comparison among studies difficult. We hope the increasing amount of information on Neotropical spiders being produced will allow us nevertheless to paint a picture of the functional relationships of the guilds, fostering the understanding of the ecosystems they live in as well.

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Colonization dynamics of agroecosystem spider assemblages after snow-melt in Quebec (Canada)

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Abstract. Spiders are important generalist predators in agroecosystems, yet early season colonization is poorly understood, especially in northern regions. We investigated colonization patterns of spiders in agricultural fields after snow-melt in four cornfields in southwestern Quebec (Canada). Paired pitfall traps were associated with two drift fences to obtain data about immigration to and emigration from the fields and were placed at increasing distances from a deciduous forest border. Control traps were placed four meters inside the forest. Seventy-four species were collected, dominated by Linyphiidae and Lycosidae. Most of the fauna was already active during the first weeks of collection, and early season assemblages differed from late season assemblages. A significant eeotone effect was found for spider abundance, species richness and species composition. This study stresses the importance of early season spider activity in agroecosystems, and this context is relevant to a period of colonization by the dominant, active spider species.

Keywords: Agroecosystems, early scason assemblage composition, dispersal, Linyphiidae, Lycosidae

Generalist arthropod predators, including spiders, are important biocontrol agents in agroecosystems (Riechert & Lawrence 1997; Symondson et al. 2002; Stiling & Cornelissen 2005) and, when seen as a species assemblage, can exert top-down effects on many agricultural pests (Riechert & Bishop 1990; Carter & Rypstra 1995). Their efficiency as pest control agents is, however, influenced by several factors including intra-guild predation (Balfour et al. 2003), cannibalism (Buddle et al. 2003), prey preference (Harmon & Andow 2004; Toft 2005) and colonization of agricultural habitats (Hibbert & Buddle 2008; Sackett et al. 2009).

Many generalist arthropod predators spend the winter in non-cultivated marginal habitats before colonizing fields in spring (Alderweireldt 1989; Thomas & Marshall 1999; Maloney et al. 2003). While marginal habitats are known to increase the diversity and abundance of generalist arthropod predators in fields (Halaj et al. 2000; Landis et al. 2000; Lemke & Poehling 2002; Schmidt et al. 2008; Sackett et al. 2009), few quantitative studies have estimated the proportions of arthropods using marginal habitats as shelters to spend the winter (Pywell et al. 2005). There is also evidence that dominant species in agricultural fields (e.g., agrobiont species: Luczak 1979; Samu & Szinetàr 2002) show synchronization with habitat changes and disturbances. In other words, species of ecological importance may spend most of their lives within disturbed habitats such as agroecosystems (e.g., linyphiids in desert agroecosystems: Gavish-Regev et al. 2008; Pluess et al. 2010) and make little use of marginal habitats as overwintering shelters (Sunderland & Samu 2000).

Early colonization dynamics of arthropod predators can be especially important in northern systems where the snow cover is extensive and active movement of predators into agricultural fields during the spring can only occur after snow-melt. Spiders are among the first predators to colonize agricultural fields (Maloney et al. 2003) and prey on numerous pest insects (Young & Edwards 1990; Pfannenstiel 2008). However, at northern latitudes, most studies of spider colonization of agroecosystems have focused on the summer

season, when spider abundanee is high (e.g., Hibbert & Buddle 2008; Sackett et al. 2008, 2009). This can bias our understanding of the way colonization proceeds in northern countries where certain taxa remain active and forage under the snow layer (e.g., Lycosidae and Lyniphiidae, Aitchison 1984 a, b).

To date, few studies have investigated early season dynamics and spider movement after snow-melt (Juen et al. 2003). Since spiders usually move into the field sooner than pests or specialist predators (Agnew & Smith 1989; Young & Edwards 1990; Marc et al. 1999), early colonization could help maintain a steady population of generalist arthropod predators, thus maximizing their affect on pests. Furthermore, early season colonization dynamics can differ greatly from those observed in late season studies: with the exception of the Linyphidae, most spider species disperse aerially while in immature stages when they can easily be lifted by air currents (Dean & Sterling 1985). Spiders usually overwinter either as adults or penultimates (Aitchison 1984a; Foelix 1996), suggesting that the cursorial mode of colonization could prevail over ballooning after snow-melt. Even though linyphiids are capable of ballooning at all stages, atmospheric conditions are unlikely to be favorable for aerial field colonization during early season. Hibbert & Buddle (2008) have also stressed the importance of cursorial movement over ballooning for short-distance colonization of cornfields.

We tested how distance to a forest-field ecotone, direction of movement and sampling week affected cornfield spider assemblages after snow-melt in southwestern Québec (Canada). The objectives of the research were to determine how spider abundance, species richness and assemblages varied temporally after snow-melt, and to compare spider abundance, species richness and assemblages as the distance to a non-managed forest border increased.

METHODS

Site description.—The study area was located adjacent to the Morgan Arboretum (Sainte Anne de Bellevue, 45.42°N, 73.95°W, Quebec, Canada). The experiment was established on four cornfields (*Zea mays*) with similar soil characteristics

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operated by the Macdonald campus farm, McGill University. These fields were on a corn-alfalfa rotation and managed using reduced tillage practices. Corn residues were left on the soil after harvesting during the previous year, and no insecticides were sprayed during the course of our study. All fields were adjacent to the Morgan Arboretum, a 254 ha deciduous reserve dominated by *Acer saccharum*, *A. rubrum* and *Fagus grandifola*. One of the studied fields was divided in two because of its large area (> 12 ha) compared to the three other fields (3 ha each). This allowed for five separate sites (i.e., five replicates) for experimentation.

Sampling method.—Distance of colonization into the field was assessed by placing paired pitfall traps at 0, 4, 8, 16, and 100 m from the forest-field ecotone plus a control trap 4 m inside the forest border. One study site was not large enough to install a trap at 100 m; the trap was thus set at half the width of the field (54 m). Since no significant differences in abundance ($\chi^2_1 = 1.64$, P = 0.2) and species richness ($\chi^2_1 =$ 0.06, P = 0.8) were found, these traps were grouped with 100 m traps for subsequent analyses. Paired traps were set at least 10 m apart from each other and spaced perpendicular to each other instead of in a linear transect, and traps within each replicates were located at least 200 m apart. To obtain data about immigration to and emigration from the field, we used two drift fences arranged in a "V" shape perpendicular to the forest border and containing a pitfall trap at the center of each fence. Traps facing the field center were counted as movement from the field into the forest, and traps facing the forest border were counted as movement into the field. The drift fence consisted in a 75 long \times 15 cm high piece of aluminum flashing embedded 5 cm deep in the ground. The pitfall traps were plastic cups (6 cm diameter×6 em height) filled with 1 cm of propylene glycol diluted 3:1 with water. Each trap was covered with a 15×15 cm plastic roof maintained about 5 cm above ground in order to avoid flooding by rain. The total trapping effort was therefore 60 pitfall traps (2 drift fences \times 6 distances \times 5 replicates).

Snow cover was extensive during the 2008 winter, since over 370 cm of snow precipitation was recorded (Environment Canada 2011). Snow-melt happened quickly, and no snow patches were observed in the field after the second week of collection. Traps were installed on 16 April 2008, immediately after snow-melt in the fields, and were collected weekly from 22 April until 2 July 2008, with a total of seven collection dates. Ecotone and field traps were removed from 7 May to 23 May for tillage-seeding period and from 6 June to 17 June for mid-season herbicide spraying. Forest traps were kept active during field disturbances and collected on 23 May (Week 5) and 17 June (Week 9) in order to see how spider abundance and species richness were affected by these disturbances. All adults collected were identified to species using Paquin & Dupérré (2003), and nomenclature followed Platnick (2011). Voucher specimens were deposited in McGill University's Lyman Entomological Museum (Ste. Anne de Bellevue, Quebec, Canada).

Statistical analyses.—We used Generalized Estimating Equations (GEE) with Poisson error and a log link to test the effects of distance, direction of traps and sampling week in R version 2.10.1 (R Development Core Team 2009) for Macintosh with the package geepack (Højsgaard et al. 2005).

Response variables included spider abundance, abundance of the two most eommon families and the three most common species. Raw species richness was positively correlated with spider abundance (Spearman $\rho = 0.91$, P < 0.0001), and we therefore only present abundance data. Individual traps were set as the repeated measure, and we used an exchangeable correlation structure since sampling events were not equally interspersed due to tillage and herbicide spraying events. Number of sampling days was used as an offset variable to correct for sampling effort. Because of small sample size, only two-way interactions were considered. Species richness was estimated for distance, direction of colonization and sampling week with the non-parametric estimators Chao 2, Jacknife 1, Jacknife 2 and Bootstrap using EstimateS version 8.2 for Macintosh (Colwell 2009). These estimators perform well in case of high occurrence of rare species and are less dependent on sample size (Magurran 2004).

To assess the effect of sampling week and distance to border (continuous variables) on spider assemblage composition, we used non-metric multi-dimensional scaling (NMDS) ordinations with the package vegan (Oksanen et al. 2010). NMDS is a non-parametric technique that does not require linear relationships between variables (McCune & Grace 2002). Singletons and doubletons were excluded from the dataset, and abundances were \log_{10} (abundance+1) transformed to decrease the influence of common species. NMDS that was run on raw abundance data showed substantially similar results and did not improve the fit of the analysis. We therefore only present results for log-transformed data. In order to minimize stress, data were pooled per distance and direction to observe sampling week effect on assemblage composition and pooled per sampling week and direction in order to observe distance effect. We ran a preliminary sixdimensional analysis to determine the optimal number of dimensions in order to minimize stress [parameters: Bray-Curtis distance measure, random starting configuration based on the time of the day, 500 iterations maximum (McCune & Grace 2002)]. We re-ran the NMDS using the same parameters as above, but altered the number of dimensions as recommended by the preliminary run and used the graph data from the initial run for starting coordinates (McCune & Grace 2002). To further analyze if differences in species composition could be observed between distance and sampling week, we used PERMANOVA (Anderson 2001, function adonis in Vegan). PERMANOVA is a non-parametric technique that assesses distance between groups based on a dissimilarity matrix (Anderson 2001) and can handle continuous predictors. We used Bray-Curtis distance to correspond with NMDS metrics and performed the permutation tests within replicates to conserve the structure of the data (n =5000 permutations). Multiple comparisons between distances and sampling weeks were performed using the software PAST version 2.11 for Windows (Hammer et al. 2001).

In order to identify agrobiont species, we used Indicator Species Analysis with the package labdsv (Roberts 2010) (function indval) on the ten most abundant species in field traps. Indicator Species Analysis compares the distribution of a given species within a group of samples to a random generation of the group (Dufrene & Legendre 1997). Traps were grouped into three habitats (forest, ecotone and field)

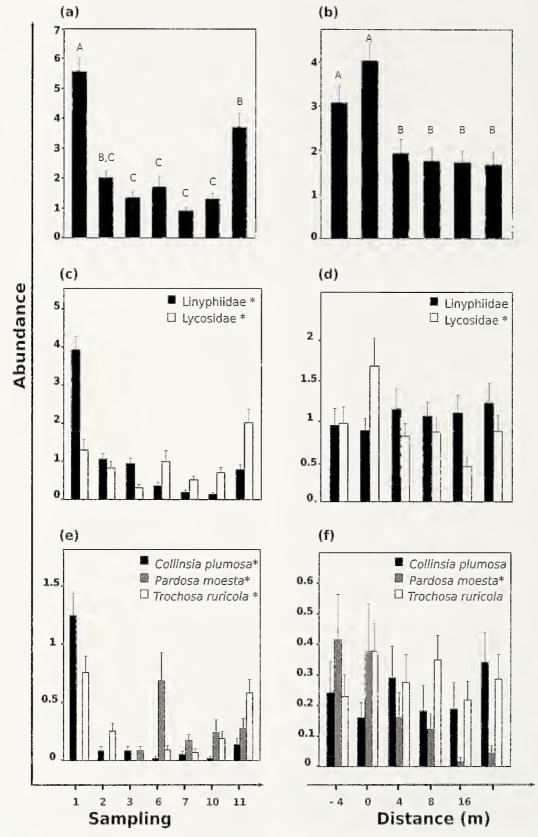


Figure 1a-f.—Mean spider abundance (+SE) in function of sampling week and distance to the forest-field ecotone. Letters indicate significantly different means at alpha = 0.05. Effects of sampling week and distance on total abundance (a, b) on dominant families (c, d) and on dominant species (e, f).

Table 1.—Species richness and richness estimators in function of sampling week (a), distance from the forest-field ecotone (b), and direction of colonization (c) (mean \pm SD).

			(a)				
Sampling week	1	2	3	6	7	10	11
Raw species richness	20 ± 3.8	19 ± 3.8	17 ± 3.6	16 ± 3.6	10 ± 2.9	11 ± 3.1	28 ± 4.4
Chao 2	28.8 ± 7.3	29.4 ± 11.4	20.8 ± 6.3	24.2 ± 6.6	17.6 ± 3.8	22.9 ± 8	41.6 ± 8.6
Jacknife 1	27.9 ± 2.3	25.7 ± 2.9	19.7 ± 2.1	22.6 ± 2.8	16.6 ± 2.1	20.8 ± 2.7	38 ± 3.9
Jacknife 2	29.4 ± 5.4	28 ± 7.4	20.9 ± 4.9	24.6 ± 6.6	17.9 ± 5.3	22.5 ± 6.5	42.1 ± 8.9
Bootstrap	25.1 ± 2.2	22.5 ± 3	17.7 ± 2	19.4 ± 2.8	14.3 ± 2.3	18.1 ± 2.7	32.8 ± 3.9
			(b)				
Distance (m)	-4	0	4	8	16	100	
Raw species richness	35.5 ± 3.7	40 ± 4.5	22 ± 3.9	16 ± 3.6	15 ± 3.5	16 ± 3.6	
Chao 2	20.6 ± 2.1	64.2 ± 5.6	47.6 ± 5	34.71 ± 3.3	39.1 ± 4.4	35.5 ± 3.7	
Jacknife 1	22.1 ± 4.8	46.2 ± 3.8	28.9 ± 2.7	22 ± 2.3	20.8 ± 2.5	20.6 ± 2.1	
Jacknife 2	18.1 ± 2	48.7 ± 8.5	31.4 ± 6.3	23 ± 5.5	22.8 ± 6	22.1 ± 4.8	
Bootstrap	30.1 ± 2.9	41.2 ± 3.4	25.3 ± 2.6	19.7 ± 2.3	18.2 ± 2.7	18.1 ± 2	
			(c)				
Direction	Forest-field	Field-forest					
Raw species richness	46 ± 4.4	41 ± 4.6					
Chao 2	55 ± 7.5	48.7 ± 6					
Jacknife 1	57 ± 4	52 ± 3.7					
Jacknife 2	61.6 ± 9.3	55 ± 8.6					
Bootstrap	50.5 ± 3.9	46.4 ± 3.7					

and three sampling periods (early season (weeks 1 through 3), post-tillage (week 6 and 7) and post-herbicide (week 10 and 11)).

RESULTS

We collected a total of 1076 individuals representing 74 species and 14 families. Of these, 25% were immature spiders and could not be identified to species level. Singletons and doubletons represented more than 50% of all captured species and the most commonly collected families were Linyphiidae (444 individuals), Lycosidae (435 individuals) and Thomisidae (103 individuals). The three most abundant species were the wolf spiders *Trochosa ruricola* (De Geer 1778) (121 individuals), *Pardosa moesta* Banks 1892 (114 individuals) and the linyphiid *Collinsia plumosa* (Emerton 1882) (97 individuals).

Spider abundance and species richness.—No effects of direction were found for all tested models (P > 0.1). Even during field disturbance weeks, no effect was reported for forest traps (P > 0.1). For the total abundance model, significant effects were found for distance ($\chi^2_5 = 78.1$, P <0.0001) and sampling week ($\chi^2_6 = 286.6$, P < 0.0001) and a significant distance × sampling week interaction was reported $(\chi^2_{30} = 126.8, P < 0.0001)$. Spider abundance was highest during the first and last week of collection, while species richness remained stable across sampling weeks except for the last collection date (Fig. 1a, Table 1a). Spider abundance and species richness decreased as distance to the forest-field ecotone increased (Fig. 1b, Table 2b). Overall, abundance and species richness were highest at the ecotone and 4 m inside the forest, whereas field traps had similar abundances and species richness. Despite the interaction between sampling

week and distance, the shape of the distance effect was relatively similar across all sampling weeks, with the exception of weeks 1 and 7 (Fig. 2). Similarly to spider abundance, direction of colonization did not affect spider estimated species richness (Table 1c).

Table 2.—*P*-values for multiple comparisons of sampling week (a) and distance (b) effects on spider assemblages using PERMANOVA. * indicates significant *P*-value at alpha = 0.05.

		and the second s		(a)			
		-	Sampl	ing week			
	1	2	3	6	7	10	11
1	-	-	-	-	-	-	-
2	0.0083*	-	-	-	~	-	-
3	0.0078*	0.47	-	-	-	-	-
6	0.0077*	0.0072*	0.0096*	-	-	-	-
7	0.0062*	0.008*	0.0065*	0.35	-	-	-
10	0.0084*	0.0099*	0.0092*	0.41	0.22	-	-
11	0.0078*	0.0078*	0.0082*	0.017*	0.0082*	0.24	-
				(b)			
			Dista	nce (m)			
		-4	0	8	10	6	100
-4		-	-	-	-		-
0	0.0)52	-	_	-		-
8	0.0	089*	0.70	-	-		-
16	0.0	019*	0.24	0.58	-		-
100	0.0	039*	0.62	0.64	0.6	55	-

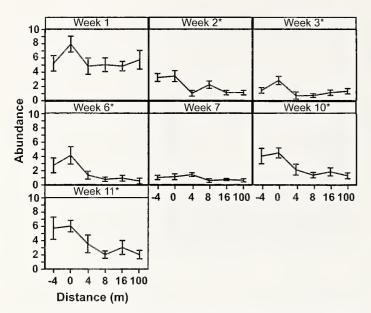


Figure 2.—Mean spider abundance (\pm SE) in function of sampling week and distance to the forest-field ecotone.* indicates significant P-value at alpha = 0.05.

At the family level, GEE analysis revealed significant family \times distance ($\chi^2_5 = 14.3$, P < 0.05) and family \times sampling week $(\chi^2_6 = 82.9, P < 0.0001)$ interactions. Linyphiids were most abundant during the first week of collection and decreased steadily afterwards while lycosids showed the opposite pattern, and distance to the ecotone only affected lycosids $(\chi^2_5 = 12.4, P < 0.05; \text{ Fig. 1c, d})$. At the species level, we also found evidence of species specific responses to the distance gradient and sampling week as the interaction terms with species were significant (distance \times species: $\chi^2_{10} = 32$, P < 0.0005; sampling week \times species: $\chi^2_{12} = 34053$, P < 0.0001). Only P. moesta was absent from the early season data set and had increased abundance as sampling went on (Fig. 1e). Collinsia plumosa appeared mostly during the first week of collection, whereas T. ruricola had high abundance on the first and last weeks of collection. Pardosa moesta was also the only dominant species to show a strong distance effect ($\chi^2_5 = 21.9$, P < 0.001), with a preference for forest and ecotone habitats and lower abundance in remote field traps (Fig. 1f).

Spider assemblage composition.—PERMANOVA on spider assemblages indicated a significant effect of sampling week $(F_{I,33} = 8.09, R^2 = 0.19, P < 0.001)$. NMDS ordination provided a two-dimensional solution that minimized stress after 105 iterations (final stress = 6.75). The three first weeks of collection showed a clear separation from later collection dates on axis 1, while later season assemblages showed strong overlap (Fig. 3a). Multiple comparisons of PERMANOVA results on sampling weeks confirmed this trend and indicated that the first week of collection differed significantly from all other sampling weeks (P < 0.01; Table 2a). Weeks 2 and 3 had similar assemblage composition as well as weeks 6, 7, 10 and 11. We hereafter refer to early season period for the three first weeks of collection, post-tillage period for weeks 6 and 7 and post-herbicide period for weeks 10 and 11.

Distance to the ecotonc also significantly affected spider assemblages ($F_{1,28} = 2.27$, $R^2 = 0.075$, P < 0.021). The

NMDS ordination produced a two-dimensional solution that minimized stress after 115 iterations (final stress = 5.75). Spider assemblages were distinct between the forest border and field traps, but showed overlap between ecotone and field habitats (Fig. 3b). Multiple comparisons of distances with PERMANOVA results showed a similar trend: the ecotone was similar to both field and forest habitats, while the forest and the field differed significantly (P < 0.01) (Table 2b).

Species that were frequently collected in field traps also had high relative abundance during the early season (first three weeks of collection) (Table 3a). Indicator species analysis did not show any species with significant affinity for the field habitat, as most species were equally associated with the ecotone and the field.

DISCUSSION

Our results indicate that spiders were active immediately after snow-melt and were frequently collected in field traps in the early spring. The forest and ecotone habitats had distinct spider assemblages, but some overlap was shown between ecotone and field habitats (Fig. 3b). This could mean that a significant proportion of the spider diversity used field habitats as shelters during the winter and mitigates the influence of surrounding non-crop habitats as sources for field colonization (Alderweireldt 1989; Thomas & Marshall 1999; Maloney et al. 2003).

The most abundant species were active quickly after snowmelt (Table 3): over 300 spiders were collected during the very first week of sampling, which represents a third of our sample size. Out of the 74 species collected, 29 were first collected during early season. Linyphiidae and Lycosidae composed most of the early spider assemblage, and two of the most common species (*T. ruricola* and *C. plumosa*) had high activity density during this period. NMDS ordination and PERMANOVA on sampling weeks confirmed that early season assemblages differed significantly from the later season. In short, the early season was composed of few species with high abundance compared to later season assemblages (Table 1a, Fig. 1a).

These results show that the dominant species and families were those active early after snow-melt and with high affinity for the field environment. Indeed, the life history of some species may be such that they spend their entire lives within disturbed habitats such as agroecosystems (Samu & Szinetàr 2002; Gavish-Regev et al. 2008; Pluess et al. 2010). According to Samu & Szinetàr (2002), the agrobiont community is typically represented by "less than 10 species making up 60 to 90% of the whole spider community". In our case, the ten most abundant species formed 68% of the field assemblage (Table 2). These species had high activity density during early season (over 50% of these species were captured during this period, except for P. moesta) and were frequently collected in field traps. Three species (Pardosa moesta, Trochosa terricola and *Diplostyla concolor*) were unlikely to be agrobiont species, since their indicator species value was low in the field habitat (Table 3). The rest of the agrobiont assemblage had equal indicator value in field and ecotone habitats, but this could be duc to low sample size. To confirm whether these species effectively use field habitats during the winter, sampling techniques enabling spider collection under snow (Paquin

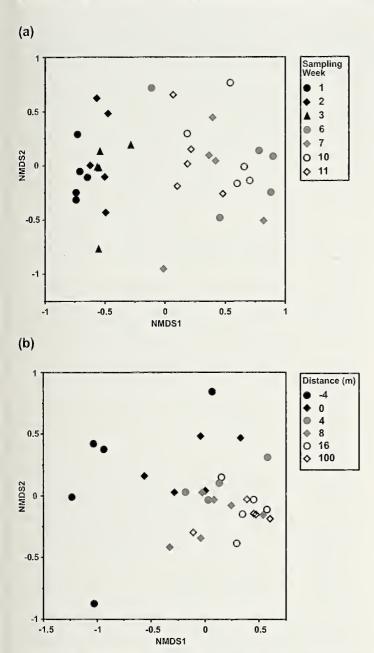


Figure 3a, b.—NMDS ordinations based on log-transformed abundance data of 35 spider species depicting spider assemblage composition in function of sampling period (a) and distance (b). Data points represent pooled samples (n = 35 for sampling week, n = 30 for distance).

2004) could provide useful information on the identity and life cycles of agrobiont spiders.

Species richness and spider abundance were both highest in the forest and ecotone habitats compared to the field. Analysis of spider assemblages at the different distances from the ecotone indicated that the ecotone was similar to the field and forest habitats. These results are confirmed by several studies on terrestrial arthropods that have documented similar increases in abundance and species richness at the ecotone compared to the two adjacent habitats (Helle & Muona 1985; Jökimaki et al. 1998; Pearce et al. 2005a; Öberg & Ekbom 2006). This effect can be explained by the fact that species that

are usually present in only one of the two habitats may meet in the overlapping ecotone (Samu et al.1999). More recent studies confirmed this fact and showed the positive influence of landscape heterogeneity and high degree of perennial crops in the surrounding landscape on spider abundance and species richness (Clough et al. 2005; Schmidt et al. 2005; Öberg et al. 2007, 2008; Gavish-Regev et al. 2008; Pluess et al. 2008, 2010). This is also supported by Juen & Traugott (2004), where spider assemblages in a small field (~ 0.3 ha) had little withinfield variation, whereas the ecotone was distinct from other sampled habitats.

The modalities of field colonization are likely to differ depending on families or even between species. Lycosid abundance decreased with distance, while linyphiids did not (Fig. 1d). Species such as T. ruricola, however, were abundant irrespective of distance, and P. moesta was highly affected by this variable (Fig. 1f). It is unlikely that this pattern was caused by aerial dispersal for lycosids, since ballooning mostly occurs at immature stages in this family (Dean & Sterling 1985; Pearce et al. 2005b) and they use cursorial dispersal as their main mode of agroecosystem colonization (Luczak 1979; Weyman et al. 2002). An early ballooning event from the surrounding landscape cannot be ruled out for linyphiids (Gavish-Regev et al. 2008). However, in temperate countries, major ballooning events occur mainly after crop seneseence rather than in the spring (Sunderland & Topping 1993; Topping & Sunderland 1994). Contrary to Lemke & Poehling (2002) where linyphiid densities were low after winter in the absence of adjacent sown weed strips, in our case linyphiid densities were high immediately after snow-melt. In an experiment conducted in the same cornfields, Hibbert & Buddle (2008) showed that field colonization occurred primarily through cursorial dispersal. We also frequently encountered active linyphiid webs after snow-melt, suggesting that linyphiids were already present in the field before snowmelt (Royauté, personal observation).

Particular attention can be given to the wolf-spider T. rmicola, the most abundant species in field traps. This largesize lycosid typically feeds on aphids, collembolans and dipterans (Kielty et al. 1999) and originated from Eurasia. It was recently introduced in the New World, potentially via the Paleartic (Platnick 1993) and was first documented in North America by Edwards (1993) then by Lalongé et al. (1997) for Canada. This species is very similar in morphology to the native species T. terricola (Thorell 1856), but has slightly different habitat preferences. Trochosa terricola is abundant in forest areas, moist meadows and forest borders, whereas T. ruricola inhabits more disturbed areas such as vegetable gardens or arable fields (Edwards 1993). There is limited evidence that T. rnricola can displace the native species, especially in perennial crops such as vineyards (Lalongé et. al. 1997; Bolduc et al. 2005). In the present context, however, T. rmicola showed little overlap with T. terricola's habitat choice since the latter was largely absent in field traps (Appendix 1). The presence of a species showing such affinity for field habitats could therefore represent a selective advantage in this particular system.

The fact that the early season showed such a distinct colonization dynamies has several implications for spiders' role in agroecosystems. As suggested by Juen et al. (2003),

Table 3.—Ten most abundant species in field traps with their indicator species values (I.V.) by habitat (Forest, Ecotone and Field traps) and sampling period (ES: early season, PT: post-tillage, PH: post-herbicide). *P*-values were calculated for the class that had highest indicator value. Bold indicates species that do not belong to the agrobiont, * indicates significant P-value at alpha = 0.05.

			Н	abitat I.V.		Sampl	ing perio	d I.V.
Species	Abundance in field traps	Relative abundance in ES (%)	Forest	Ecotone	Field	ES	PT	PH
A.fabra (Linyphiidae)	16	62.5	0.02	0.22	0.25	0.26	0.16	0.026
A. jacksoni (Linyphiidae)	17	76	0.06	0.29	0.16	0.42*	0.09	0.005
C. plumose (Linyphiidae)	76	87	0.005	0.5*	0.43	0.65*	0.06	0.02
D. concolor (Linyphiidae)	6	83	0.07	0.56*	0.033	0.4*	0.009	0.034
E. atra (Linyphiidae)	21	43	0.09	0.09	0.28	0.11	0.38*	0.02
I. flaveola (Linyphiidae)	51	98	0.04	0.47	0.41	0.72*	0	0.002
W. spiralis (Linyphiidae)	31	87	0	0.26	0.42	0.67*	0.67	0
P. moesta (Lycosidae)	22	0	0.61	0.2	0.031	0	0.33	0.53*
T. ruricola (Lycosidae)	80	54	0.14	0.44	0.31	0.39	0.46	0.05
T. terricola (Lycosidae)	10	60	0.07	0.36	0.054	0.33	0.07	0.008

spider species that are active early in the season can represent an important first line of defense against pests. Though many pests use non-crop habitats to spend the winter (Morishita 1992; Norris & Kogan 2005), some pests such as the European corn borer overwinter in corn stalks (Coll & Bottrell 1991). Given that the field habitat has no vertical structure after snow-melt, pest overwintering in the field could be more vulnerable to spiders even when still in a diapause state. Pfannenstiel (2008) showed that some families (Linyphiidae, Lycosidae) prey on lepidopteran eggs, and it is possible that spiders are capable of consuming prey that are in a diapause state. Alternatively, cannibalism and intraguild predation could be particularly important during the early season period since the diversity and density of prey may be low. Further investigations of species interaction during early season would be required to shed light on these questions.

Our results indicated that spiders were captured directly after snow-melt, forming an important potential natural enemy complex in early season, mostly composed of liny-phiids and lycosids. The forest border and ecotone habitats had higher abundance and species richness than the field, but the ecotone showed overlap with field assemblages. The most abundant species were active quickly after snow-melt and were frequently collected in field traps. In this respect, early season was the period when most of the spider species of agronomical value were active, and early season could be important in facilitating high abundance of spiders in arable fields.

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Appendix 1.—Number of individuals collected per distance to border (-4, 0, 4, 8, 16, 100) and sampling period pooled for all replication (ES: Early Season, T: Tillage, PT: Post-Tillage, H: Herbicide spraying, PH: Post Herbicide). * indicates species belonging to the agrobiont.

	-4	0	4	8	16	100	ES	Т	РТ	Н	PH	Total
Agelenidae												
Agelenopsis sp.	0	1	0	1	0	0	0	0	0	0	2	2
Corinnidae												
Plurinotimpus alarius (Hentz 1847)	3	0	0	0	0	0	0	0	1	0	2	3
Plantotimpus borealis (Emerton 1911)	4	0	0	0	0	0	0	0	0	1	3	4
Scotinella divesta (Gertsch 1941)	0	1	0	0	0	0	0	0	0	0	1	1
Scotinella pugnata (Emerton 1890)	1	2	0	0	0	1	2	0	0	0	2	4
Clubionidae												
Clubiona abbotti L. Koch 1866	0	0	0	0	1	0	0	0	0	0	1	1
Clubiona bishopi Edwards 1958	1	0	0	0	0	0	0	1	0	0	0	1
Clubiona canadensis Emerton 1890	1	0	0	0	0	0	0	0	1	0	0	1
Chibiona riparia L. Koch 1866	0	3	0	0	0	0	0	0	1	0	2	3
Clubionidae sp.	2	1	0	0	1	0	3	0	1	0	0	4
Dictynidae												
Cicurina arcuata Keyserling 1887	2	0	0	0	0	0	1	0	0	0	1	2
Cicurina brevis (Emerton 1890)	4	0	2	1	0	0	7	0	0	0	0	7
Cicurina pallida Keyserling 1887	2	0	0	0	0	0	0	1	0	1	0	2
Emblyna sublata (Hentz 1850)	0	0	2	0	0	0	0	0	2	0	0	2
Gnaphosidae												
Drassylus socius Chamberlin 1922	0	1	0	0	0	0	0	0	0	0	1	1
Gnaphosa orites Chamberlin 1922	0	1	0	0	0	0	1	0	0	0	0	1
Micaria pulicaria (Sundevall 1831)	0	1	0	0	0	0	0	0	0	0	1	1
Zelotes fratris Chamberlin 1920	1	4	1	0	0	0	2	0	2	0	2	6
Gnaphosidae sp.	0	1	0	0	1	0	0	0	0	0	2	2
Hahniidae												
Neoantistea agilis (Keyserling 1887)	0	1	0	0	0	1	1	0	0	0	1	2
Neoantistea magna (Keyserling 1887)	0	0	2	0	0	0	0	0	0	0	2	2
Linyphiidae												
Agyneta sp.	0	1	0	0	0	0	1	0	0	0	0	1
Agyneta fabra (Keyserling 1886)*	1	3	5	1	4	6	13	0	2	0	5	20
Agyneta jacksoni Braendegaard 1937*	2	6	2	7	4	4	18	0	1	0	6	25
Agyneta unimaculata (Banks 1892)	0	1	3	0	0	2	6	0	0	0	0	6
Baryphyma trifrons affine (Schenkel 1930)	0	0	0	0	1	0	1	0	0	0	0	1
Batyphantes brevis (Emerton 1911)	0	1	0	0	0	0	1	0	0	0	0	1
Batyphantes pallidus (Banks 1892)	1	0	0	0	0	0	0	0	0	0	1	1
Centromerus cornupalpis	20	2		1	1	1	22	2	,	0	0	26
(O. PCambridge 1875)	20 0	2 1	1	$\frac{1}{0}$	$\frac{1}{0}$	1	23 1	2 0	1	0	0 1	26 2
Centronierus furcatus (Emerton 1882) Centromerus persolutus	U	1	1	U	U	0	1	U	U	U	1	2
(O. PCambridge 1875)	1	0	1	0	0	0	1	0	1	0	0	2
Centromerus sylvicatus (Blackwall 1841)	i	i	0	0	0	0	2	0	Ô	0	0	2
Ceraticelus laetus (O. PCambridge 1874)	1	0	0	0	0	1	0	0	1	1	0	2
Collinsia plumosa O.PCambridge												
1913*	1	20	12	22	22	20	84	0	4	0	9	97
Diplocephalus cristatus (Blackwall 1833)	4	1	0	1	1	0	6	0	0	1	0	7
Diplostyla concolor (Wider 1834)	2	8	2	3	0	1	13	0	2	0	1	16
Erigone atraBlackwall 1833*	2	2	7	3	9	2	9	0	2	0	14	25
Erigone autumnalis Emerton 1882	0	1	0	2	1	1	1	0	0	0	4	5
Erigone blaesa Crosby & Bishop 1928	0	0	0	0	0	l	1	0	0	0	0	1
Grammonata gentilis Banks 1898	0	0	0	0	0	1 2	1	0	0	0	$\frac{0}{0}$	1
Grammonata gentilis Banks 1898 Islandiana flaveola (Banks 1892)*	0 3	0 14	0 9	0 9	0 15	3 18	2 67	0	1 1	0	$0 \\ 0$	3 68
Lepthyphantes intricatus (Emerton	3	14	9	9	13	10	07	U	1	U	U	08
1911)	0	1	1	0	0	0	0	0	1	0	1	2
Meioneta amersaxatilis (Saaristo &	Ü	•	•	Ü	J	J	Ü		•	J	•	
Koponen 1998)	1	0	0	0	0	0	0	0	0	0	1	1
Neriene clathrata (Sundevall 1830)	2	2	0	0	0	0	3	0	1	0	0	4
Oedothorax sp.	0	0	1	0	0	0	1	0	0	0	0	1

Appendix 1.—Continued.

COUNTY OF THE PARTY OF THE PART	-4	0	4	8	16	100	ES	Т	PT	Н	PH	Total
Oedothorax montifer (Emerton 1882)	0	0	0	0	1	0	1	0	0	0	0	1
Perregrinus deformis (Tanasevitch 1982)	1	2	1	1	1	0	5	1	0	0	0	6
Sciastes dubius (Hackman 1954)	1	0	0	0	0	0	0	1	0	0	0	1
Tenesselum formica (Emerton 1882)	0	0	0	1	1	2	0	0	0	0	4	4
Tenuiphantes zebra (Emerton 1882)	14	14	3	1	0	1	26	2	4	0	1	33
Vermontia thoracica (Emerton 1913)	1	0	0	0	0	0	1	0	0	0	0	1
Walckenaeria spiralis (Emerton 1882)*	0	6	5	5	12	9	31	0	6	0	0	37
Linyphiidae sp.	6	12	3	11	8	3	34	0	3	0	6	43
Liocranidae												
Agroeca ornata Banks 1892 Liocranidae sp.	1	0	0	0 0	0	0	0	1 0	$\frac{0}{0}$	0	0 1	1
Lycosidae	-		Ü									
Pardosa sp.	3	0	0	1	0	0	4	0	0	0	0	4
Pardosa modica (Blackwall 1846)	1	2	1	0	ő	ő	3	ő	ĭ	ő	0	4
Pardosa moesta Banks 1892	60	32	11	5	4	2	0	15	48	18	33	114
Pardosa xerampilina (Keyserling 1877)	0	2	0	0	0	0	0	0	1	0	1	2
Pirata sp.	ĺ	0	0	0	0	0	0	0	0	0	1	1
Pirata aspirans Chamberlin 1904	i	0	0	0	0	0	0	0	0	0	1	1
Pirata minutus Emerton 1885	3	5	1	0	1	0	0	0	0	0	10	10
Pirata piraticus (Clerck 1757)	0	0	0	0	1	0	0	0	0	0	1	1
Pirata zelotes Wallace & Exline 1978	1	0	0	0	0	0	0	0	0	0	1	1
Schizocosa communis (Emerton 1885)	1	0	2	0	1	0	0	0	3	1	0	4
Schizocosa crassipalpata Roewer 1951	0	3	0	0	0	0	0	0	1	0	2	3
Trochosa sp.	2	5	6	2	2	3	7	0	7	1	5	20
Trochosa ruricola (De Geer 1778)*	14	27	23	19	10	28	65	0	9	2	45	121
Trochosa terricola Thorell, 1856	2	7	3	6	1	0	14	0	1	1	3	19
Lycosidae sp.	43	47	16	10	12	5	51	8	14	1	59	133
Mimetidae												
Ero canionis Chamberlin & Ivie 1935	0	2	0	0	0	0	2	0	0	0	0	2
Mimetus eperoides Emerton 1882	0	0	1	0	0	0	0	0	0	0	1	1
Philodromidae												
Thanatus striatus C. L. Koch 1845	0	1	0	1	0	0	0	0	2	0	0	2
Tibellus maritimus (Menge 1875)	0	1	0	0	0	0	0	0	0	0	1	1
Tibellus oblongus (Walckenaer 1802)	0	1	0	2	0	0	0	0	0	0	3	3
Philodromidae sp.	4	0	2	1	2	1	7	0	2	1	0	10
Pisauridae						0	0	0	0		0	
Dolomedes triton (Walckenaer 1837)	1	0	0	0	0	0	0	0	0	1	0	1
Tetragnathidae								_				
Pachygnata autumnalis Marx 1884	0	1	0	0	0	0	0	0	0	0	1	1
Pachygnata xanthostoma C. L. Koch 1845	2	5	0	2	0	0	8	0	0	0	1	9
Theridiidae												
Crustulina sticta (O. PCambridge 1861)	0	1	0	0	0	0	1	0	0	0	0	1
Robertus spinifer (Emerton 1909)	1	0	0	0	0	0	0	0	0	0	1	1
Theridiidae sp.	1	0	0	0	0	0	1	0	0	0	0	1
Thomisidae												
Ozyptila sp.	1	1	0	0	0	0	0	0	1	0	1	2
Ozyptila distans Dondale & Redner									0			10
1975	11	6	0	2	0	0	0	0	0	4	15	19
Ozyptila praticola (C. L. Koch 1837)	59	3	1	0	0	0	0	2	14	19	28	63
Xysticus sp.	1	0	0	0	0	0	0	0	1	0	0	1
Xysticus elegans Keyserling 1880	3	6	2	0	0	0	0	0	4	3	4	11
Xysticus ferox (Hentz 1847)	2	3	0	0	1	0	0	0	1	2	3	6
Thomisidae sp.	1	0	0	0	0	0	0	0	0	1	0	1
Unknown	1	1	0	0	0	0	1	0	0	0	1	2

Intensive grazing opens spider assemblage to invasion by disturbance-tolerant species

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Abstract. Grazing is an established conservation tool for maintaining grassland habitats and under some circumstances may enrich arthropod assemblages. However, even if enrichment occurs, it is not granted that conservation value signified by rare and specialist species will also increase. To assess how some preset levels of grazing suit conservation aims, we studied spider assemblages of ungrazed, sparsely grazed and intensively grazed areas of a pasture in Hungary for three years by pitfall trapping and suction sampling. At ground level there was no significant difference among grazing areas, while at higher strata increasing grazing intensity negatively affected number of individuals and species. C-score analysis indicated equally neutral community assembly in all three grazing areas. All statistical methods that took into account species identity indicated virtually no difference between the spider assemblages of the sparsely grazed and ungrazed areas; however, there was a marked difference between these and the intensively grazed area. Spider species in the intensive grazing area had significantly lower affinity but wider tolerance for habitat naturalness, preferred more open habitats and had a lower rarity status. In the intensive grazing area a number of disturbance-tolerant species, among them agrobionts, were present, whereas the exclusion of rare or specialist species in the intensively grazed area occurred infrequently. The primary effect seen at the intensive grazing area was the opening of the spider assemblage to disturbance-tolerant species, while species richness was likely maintained by neighboring source populations. Overall, we experienced a marked decrease in the naturalness status of the spider assemblage in the intensive grazing area.

Keywords: Species richness, neutral community, rare species, grassland, agrobiont, trait-based assessment, Araneae

Grazing is a naturally occurring ecosystem process, which can be part of agricultural production, and recently it has also become a management tool for nature conservation. Its impact on the vegetation has been widely studied (e.g., Belsky 1992; Adler et al. 2001), in both vertebrates (Baldi et al. 2005) and invertebrates, including spiders (Gibson et al. 1992; Bonte et al. 2000; Horvath et al. 2009). Grazing can be of many kinds and may affect ecosystems in variable ways. An increase in plant species diversity and spatial heterogeneity has been reported due to the preferential grazing of the dominant grasses and concomitant increases in subordinate species (Hartnett et al. 1996). Others report both increases and decreases in plant diversity attributable to grazing in different communities (Belsky 1992), which might be caused by the different scales of the studies (Kohyani et al. 2008). Spider communities of grazed habitats also show varied responses, which range from 'virtual extinction' (Thomas & Jepson 1997), to a moderate decrease in diversity (Abrous Kherbouche et al. 1997) and the preservation of rare and specialist species (Zulka et al. 1997). Recent Hungarian studies have indicated that grazing created relatively strong local changes in vegetation structure and height, which were more important in shaping spider communities than larger scale factors such as fragmentation or landscape neighborhood (Batary et al. 2008; Horvath et al. 2009). However, in other systems local grazing effects have been overridden by landscape scale factors (Harris et al. 2003).

The effect of grazing gains special importance if we consider it from a conservation point of view. Moderate grazing may fall into the category of intermediate disturbance (Connell 1979), which is known to produce high diversities at patch scale (Whittaker et al. 2001). Moderate grazing in interaction

with succession may result in high diversity, because grazing prevents the climax stage, arresting succession at a stage when species diversity is high, as has been proven, for instance, in grasslands of the Carpathian Basin (Ruprecht 2005). The interaction between vegetation succession and grazing is also important at regional scales, where grazing is a major force that maintains grassland areas and prevents homogeneous afforestation, which would be the norm for the largest part of Central Europe. The pollen record suggests that domestic grazing has formed the landscape in Hungary since the Bronze Age (Chapman et al. 2009). As such, maintaining the "right level" of grazing should be a priority for any conservation strategy that aims to maintain biotopes integrated with traditional human activities.

To judge the effect of grazing is not simple. Even though in some situations grazing might contribute to the increase of species richness, that in itself does not guarantee an increase in conservation value; for example, if a natural habitat is disturbed, then the first occurrence of a weed species will result in an increase in richness. Therefore, it is important to judge the effect of grazing on invertebrate (and other) assemblages by assessing how species interactions are affected and by weighting changes with species traits.

Periodic or constant disturbances may disrupt species interactions and may make the coexistence of a wider range of species possible, but may also increase the probability of species invasions (Hobbs & Huenneke 1992). Under grazing pressure assemblages will be more determined by their suitability to the habitat than by their competitive potential. In other words, grazing, for instance by physical perturbation and by creating spatio-temporal patchiness, may prevent species interactions (e.g., competition, intra-guild predation)

from playing a major role in the formation of assemblages. The absence of such interactions and the prevalence of stochastic processes (immigration, birth, death) in community assembly are emphasised by neutral community models. Whether grazing shifts spider communities toward neutrality has not been studied so far.

Habitats with different grazing profiles/histories are likely to have assemblages with characteristic species representing different ecological traits and tolerances. It is known that stable natural habitats harbor less dispersive specialized species (Bell et al. 2001; Bonte et al. 2004a), while ephemeral habitats—both natural and human-created, e.g., arable fields—also have very specific, disturbance-tolerant species assemblages (Samu & Szinetár 2002). Species can be ranked with respect to their preference for stable versus disturbed habitats. Such ranking has been shown to be correlated with rarity (Samu et al. 2008) and can be used for the evaluation of conservation value, both of the habitat and the spider assemblage.

In the present study we evaluate spider assemblages at three areas of different grazing levels. 1) We consider general quantitative properties of spider assemblages in the grazing areas, including abundance and species richness. 2) We ask how species coexistence patterns are affected by grazing. We hypothesize that grazing weakens species interactions; therefore, under heavier grazing assemblages will be more neutral. 3) We investigate the concrete nature of assemblage changes in the different grazing areas. Which species, of which foraging strategies, and of which kinds of ecological tolerances can adapt best to the varied grazing levels? And, would assemblages found represent different conservation values?

METHODS

Study area.—The study area was a dry pasture (47° 26′E, 18° 29′N) near Vértesboglár, Hungary (Fig. 1). The area, at an average elevation of 200 m a.s.l., lies at the feet of the Vértes Mountains, a low dolomitic range. This area is at the meeting point of closed forests and steep rock steppe habitats of the Vértes Mountains and wetland areas of the Zámolyi Basin. The original vegetation was forest-steppe mosaic, but it has been used for pastoral farming for hundreds of years. Botanically it can be described as dry grassland of average plant diversity. In this system grazing is the primary factor that maintains the grassland. Without grazing the area would be reforested, and several protected grassland species would lose their habitat.

The pasture studied was 270 ha in area. Since June 2006 the pasture has been grazed by the traditional Hungarian sheep variety "rackajuh". Because of grazing, the grassland vegetation was more homogeneous than the natural grasslands of the mountain slopes. According to vegetation height and vertical stratification two main zones could be identified, which resulted from different grazing pressures affecting the respective areas.

To study the short-term effect of different levels of grazing, we studied spider assemblages in three areas of the pasture (Fig. 1) with different grazing levels. 1) Intensively grazed area. This area of the pasture was directly eonnected to the sheepfold. The average height of the vegetation was 3–4 cm. Due to their daily activity animals spent more time here;

grazing pressure was therefore high. 2) Sparsely grazed area. Animals spent less time in this zone of the pasture and consequent grazing pressure was much lower than in the intensively grazed area. The average height of the vegetation was 5–6 cm. 3) Control, ungrazed area, where livestoek were excluded by fencing surrounding a 0.13 ha area, located in the sparsely grazed zone. Fencing was established in 2006. The average height of the vegetation was 10 cm. Sampling locations in the sparsely and intensively grazed areas were ca. 500 m apart. Sinee each grazing area had only one continuous site, interspersed spatial replication was not possible. Each grazing level was represented by one area, where the samples were taken.

The study was carried out between 13 April 2007 and 28 September 2009 during the early summer and autumn samplings. In all three areas spiders were collected by pitfall trapping and suction sampling. Catches from one trap or one suction sampling transect during a campaign are referred to as subsamples. Subsamples from one area and given sampling campaign constituted a sample. Exact timing, trap opening times and number of subsamples per area are listed in Table 1.

For pitfall trapping we used plastic eups of 75 mm upper diameter, filled with 70% ethylene glycol as preservative and some detergent (Kádár & Samu 2006). We used "Vadóc" game repellent hung on strings above the pitfalls at ca. 60 cm height to prevent domestie and wild grazing animals from demolishing the traps. We also used a hand-held motorized suction sampler to collect spiders (Samu & Sárospataki 1995) during one sampling period (Table 1). The applied suction sampler collected from an area of 0.01 m². Ten such applications in a short transect, from a cumulative area of 0.1 m², comprised one subsample.

Data analysis.—Spiders were determined using available keys (e.g., Nentwig et al. 2010); nomenclature is according to Platnick (2010). Statisties regarding spider abundance were based on standardized catches: number of spider individuals caught in a subsample per unit sampling duration (one for suction samples; number of days a trap was open for pitfalls). Statistics requiring species level information were restricted to adult spiders, determined to species level.

Species characteristics were quantified in part from the catalogue by Buchar and Růžička (2002). Using the database of the catalogue we assigned ordinal values to species characters that eould be ordered: 1) extent of distributional area, 2) preference for elevation, 3) preference for habitat naturalness, 4) preference for habitat humidity, 5) preference for light (habitat openness), 6) vulnerability status of the species and 7) frequency of occurrence. In the case of many species more than one value is listed for the four preference type characters (2–5) in the database, and some of these values were reported to be "typical" or "non-typical". To deal with this, we calculated the mean value of the character, which was either the single ordinal character value for the species, or the mean of the ordinal values in the list or, if typicality was indicated, we applied a typicality weight of 2× (for typical) or 0.5× (for non-typical) in the calculation of the mean. We also calculated the width of the preference-type characters, which was the difference between the largest and the smallest ordinal character values. As an additional species character we calculated Global Abundance Value (GAV), a species

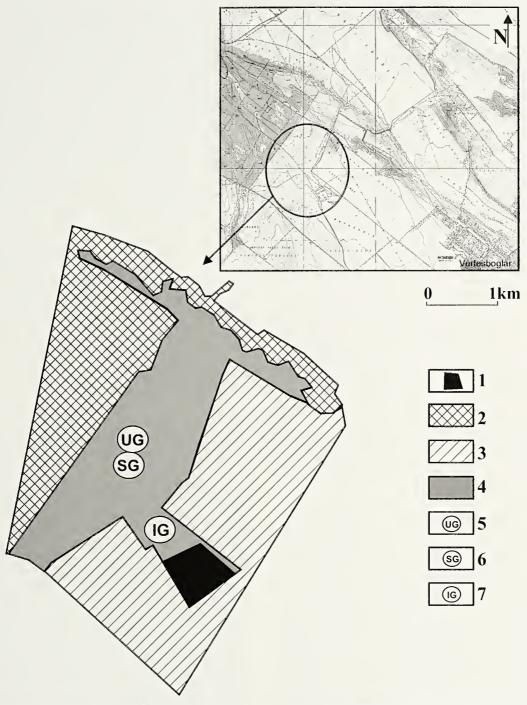


Figure 1.—Arrangement of grazing areas and sampling locations in the study pasture at Vértesboglár 2007–2009. 1, Farmyard and sheepfold; 2, Woodlands; 3, Arable land; 4, Pasture; 5, Ungrazed (= UG); 6, Sparsely grazed (= SG); 7, Intensively grazed (= IG) sampling areas.

Table 1.—Sampling efforts and timing at the three grazing areas. P = pitfall, $S = Suction sample \times number of subsamples$.

Campaign date	Traps open (days)	Exclusion control	Sparsely grazed	Intensively grazed
23.05.2007	40	$P \times 5$	P × 5	
28.09.2007	27	$P \times 5$	$P \times 5$	
08.06.2008	46	$P \times 5$	$P \times 5$	$P \times 10$
21.10.2008	27	$P \times 5$	$P \times 5$	$P \times 5$
06.06.2009	36	$P \times 5$	$P \times 5$	$P \times 5$
28.09.2009		$S \times 10$	$S \times 10$	$S \times 10$

abundance value which is an inverse measure of species rarity. It gives the proportion that individuals of a given species represent out of all individuals in a 'global' background database, which in this case was the Hungarian arachnological database. We included this value, because it has proven to be the best surrogate measure for conservation value in two case studies (Samu et al. 2008).

Following the literature (e.g., Gotelli 2000) we hypothesized that in assemblages structured by biotic interactions the presence of certain species will exclude the presence of others, generating recognizable coexistence patterns, while in neutral communities coexistence patterns generated by such interactions will be weaker or non-existent. We investigated the neutrality of species co-occurrence in the spider assemblages of the grazing areas using Stone and Roberts' C-score analysis (1990). C-score refers to the average number of "checkerboard units" (i.e., no co-occurrence situations) between all possible pairs of species. High C-score values indicate species segregation in a community. C-scores were calculated by samples (n = 16: see Table 1), considering subsamples as the units where co-occurrences were recorded. C-score analysis was executed by the program EcoSim 7.72 (Gotelli & Entsminger 2010), which constructs null models by simulation to calculate whether an observed C-score is significantly larger than can be expected by chance. In null-model construction the "sites (= subsamples) equiprobable" and "species fixed" options were used. We compared C-scores between samples using standardized effect sizes, the deviation of the observed C-score from the mean of simulated C-scores scaled to standard deviations, to make comparisons among different samples/assemblages. An effect size greater than 1.96 or less than -1.96 is statistically significant at P = 0.05 (Gotelli & Entsminger 2010).

Differences between catches and taxon numbers in the grazing areas were tested by the LME4 package in R (Bates et al. 2011) for generalized linear mixed models. We reached a final model after manual variable selection based on AIC, initially regarding year and subsample as random variables and grazing area as a fixed variable. Species characters were screened in a Canonical Correspondence Analysis (CCA). The similarity of assemblage structures was depicted by Nonmetric Multidimensional Scaling (NMS), and the significance of the differences in species composition between the revealed groupings was tested with Multi-Response Permutation Procedure (MRPP) (Mielke et al. 1976). Species that most characteristically represented the groups were shown by Indicator Species Analysis (ISA) (Dufrene & Legendre 1997). The four latter methods were applied using PC-ORD v. 5.31 (McCune & Mefford 2006).

RESULTS

During the study 1664 individuals were caught, of which 1159 were adults. Apart from the identifiable 63 species we could further identify 11 unique taxa (e.g., juveniles of genera where no adults were found); thus, the total number of taxa shown from the pasture was 74, over 10% of the species on the Hungarian check list (Samu & Szinetár 1999). See the Appendix for a complete list of catches by grazing areas.

Overall we found lower numbers of individuals and taxa where the intensity of grazing was higher (see Appendix).

Considering pitfall trap catches, neither the total number of spiders eaught (Fig. 2a) nor the number of spider taxa (Fig. 2c) differed significantly between the grazing areas. However, in suction samples both spider abundance and the number of taxa were significantly lower in the intensively grazed area than in the other areas (Figs. 2b–d, Table 2).

Considering species co-occurrences in the spider assemblages at the three grazing areas, we could detect neither species segregation, nor aggregation in the assemblage structures. None of the C-score analyses (n=16) showed significant deviation from the fixed-equiprobable null model (Gotelli 2000), and effect sizes also indicated neutral community organization in all three grazing areas. Effect-sizes among grazing areas did not differ statistically (one-way ANOVA: $F_{2.13}=1.22$, P=0.3).

Although assemblages in the different grazing areas all proved to be neutral in terms of species co-occurrence, species compositions in the intensively grazed area were different from that of the sparsely-grazed or ungrazed areas in both years (Fig. 3, MRPP difference between the arising two groups [intensive vs. (sparse + control)] for 2008: T = -7.374, P < 0.0001; for 2009: T = -8.489, P < 0.0001). The ordination plot from the NMS analysis also reveals that, in the summer samples of both years, sparsely grazed subsamples and control subsamples did not separate as distinct groups (Fig. 3).

The other ordination method, CCA, made a grouping of the samples very similar to the NMS result. In the CCA plot, samples of the intensively grazed area were placed apart from the group of control and sparsely grazed samples (Fig. 4). The pairing of samples by study year is observable in the control + sparse grazing group, underlying that difference between 'sparse grazing' and 'control' was a mild effect compared to the effect of 'year'. The distinct separation of samples from the intensive grazing area, on the other hand, shows that intensive grazing creates a much stronger difference than yearly variation or sparse grazing. The CCA depicted samples in the species space. The ordination was not constrained as usual by a second matrix of environmental variables, but by the matrix of species characters. The separation of intensively grazed samples was related to light (habitat openness) preference and habitat naturalness preference of the species, as shown by the highest inter-set correlations; i.e., the correlations between species character variables and the ordination axes 1 and 2, weighted by the eigenvalues of those axes (naturalness mean: $r_1 = 0.417$, $r_2 = 0.649$; light preference mean: $r_1 = -0.745$, $r_2 = 0.005$).

Apart from species preference for habitat naturalness and openness as chief main faetors, CCA also identified that abundance, width of preference for naturalness, and humidity are also important characters along which spider assemblages of different grazing areas differ from each other. We have tested for the significance of all these characters and found highly significant differences between grazing areas for all of them (Fig. 5). A post-hoc test indicated that the intensively grazed area's spider assemblage was the one that differed from the other two for all characters (Fig. 5).

Finally we wanted to identify which families and species are mostly responsible for the separation of the assemblages in the intensively grazed vs. ungrazed or sparsely grazed areas.

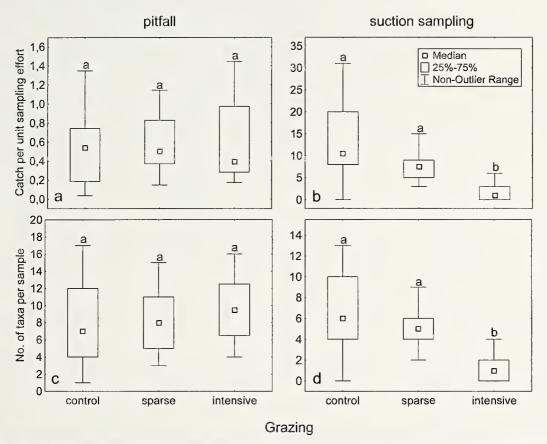


Figure 2.—The effect of sampling method and grazing intensity on number of individuals caught per unit sampling effort (a, b), and on the number of spider taxa caught per sample (b, c). Groups denoted with the same letter are not significantly different at the P = 0.05 level under Tukey HSD test.

Considering differences at the family level, Hahnidae had preference for the less-grazed or ungrazed areas, while Linyphiidae and Thomisidae had significant preference for the intensive grazing area (Table 3). An ISA was conducted at the species level to reveal the affinity of species to the grazing areas (Table 4). Over twice as many species were significant indicators of the intensive grazing area than of the less-grazed or ungrazed areas. In the Lycosidae, for instance, the larger Alopecosa species had a clear preference for the less grazed areas, while many of the smaller lycosids (Pardosa and Xerolycosa spp.) were more numerous in the intensively grazed areas. Certain 'disturbance tolerant' species [e.g., Ostearius melanopygius (O.P-Cambridge 1879)] and a number of 'agrobiont species' that are strongly associated with arable fields (Samu & Szinetár 2002) were among the indicators of the intensive grazing area (see species marked in Table 4).

Table 2.—Result of Generalized Linear Mixed Models of spider catches and taxon numbers. The models were executed separately by sampling methods, grazing was ordinal fixed variable, sampling date (in case of pitfalls) and subsample were entered as random variables. Poisson error structure and log link function was used. For the overall effect of grazing χ^2 statistics is reported.

Variable	Method	d.f.	χ^2	P
Taxon number	suction	2	13.37	0.0013
Catch	suction	2	17.40	0.0002
Taxon number	pitfall	2	0.93	0.629
Catch	pitfall	2	0.72	0.697

DISCUSSION

Our survey found that spider assemblages in the ungrazed and sparsely grazed areas had similar spider abundance and species richness, while these measures of spider assemblages were lower in the intensively grazed part of the pasture. Since grazing livestock remove biomass from pasture ecosystems, they can produce a negative cascading effect for arthropod populations along the entire food web (Hobbs 1996; Boyer et al. 2003). Grazing also removes microhabitats, with similar negative effects (e.g., Hutchinson & King 1980). Both these processes are likely to result in lower spider density, and species richness is also likely to follow this pattern of spider abundance (Bell 2000).

In the present experiment two methods were used: suction sampling is more geared toward species living in higher strata of the grass; pitfall trapping more toward species at the ground surface. Since suction sampling catches spiders with higher efficiency from the higher strata in the grass, if the volume of this stratum becomes smaller due to grazing, a decrease in catches can be expected (Greenstone 1984). Pitfall catches, unlike suction samples, showed no significant difference between the grazing areas. Grazing means not only physical disturbance but also altered trophic relationships (Meyer & Reinke 1996). In pasture soil fauna, the subsidy from the manure of grazing animals might compensate for reduced higher strata productivity (Rypstra & Marshall 2005); hence, there is a likely interaction between disturbance and productivity (Bonte et al. 2004b; Svensson et al. 2010). Thus, in an indirect way, differences between the pitfall trap and suction

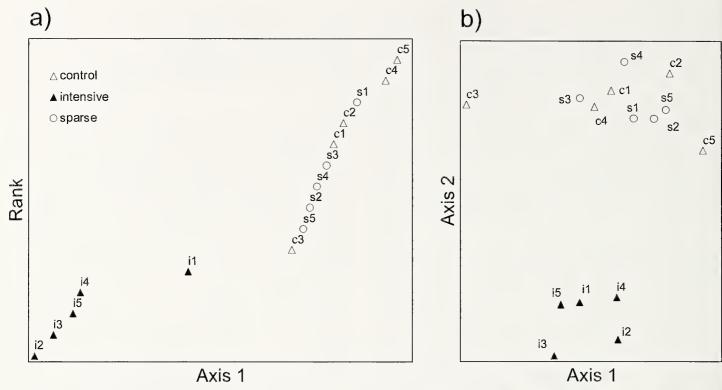


Figure 3.—NMS plots of the spider assemblages from pitfall trap catches in the three grazing areas; note how control grazing points envelop sparse grazing points in both plots. Analyses were done by PC-ORD v. 5.31 with NMS autopilot "thorough" option, Bray-Curtis distance measure. a) 2008 summer (from intensive grazing area only pitfalls 1–5): final stress S = 24.63 (one-dimensional solution is the best), Monte Carlo probability of obtaining smaller stress P = 0.008; b) 2009 summer: S = 10.91 (two-dimensional solution is the best), P = 0.004.

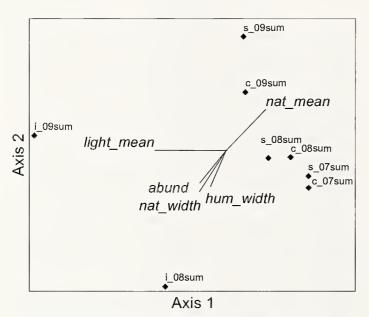


Figure 4.—Canonical Correspondence Analysis plot of yearly pitfall trap samples during the summer trapping period in the three grazing areas, constrained by a second matrix of species characteristics. See text for the explanation of how species character variables were derived. Abbreviations: abund = abundance (GAV), nat = naturalness, sum = summer; 0x = year, c = control, s = sparse grazing, i = intensive grazing. Eigenvalue $\lambda_{axis1} = 0.406$, P = 0.017; $\lambda_{axis2} = 0.081$; Samples-species characters correlation $r_{axis1} = 0.813$, P = 0.025; $r_{axis2} = 0.501$.

sampler catches underline the importance of vegetation height/volume as a predictor of species richness and abundance (Kruess & Tscharntke 2002; Schwab et al. 2002), and show that different process might act in different strata.

Little is known about how disturbances affect species interactions and assembly; specifically, the effect of grazing on invertebrate species co-occurrence is virtually unknown. Communities may show non-random species co-occurrence patterns as a result of competitive interactions (Ulrich & Gotelli 2007), but in spiders such interactions can seldom be classified as exploitative competition (Wise 1993). More often they take the form of direct interactions, such as intraguild predation and cannibalism (Samu et al. 1999; Wise 2006). Recently there have been a few studies that indicate the disruption of non-random community structure in invertebrate groups by disturbances other than grazing, such as fire (Sanders et al. 2007; Pitzalis et al. 2010) or tourism (Ulrich et al. 2010). By analogy, we expected that with stronger grazing more neutral co-occurrence patterns would emerge. However, C-score analysis suggested no deviation from neutral species assembly in any of the grazing areas. We suggest that in the grassland systems studied, neutral communities and fairly species-rich assemblages are the norm; while climax, low diversity, highly structured spider assemblages may be non-existent, in part because some level of disturbance (e.g., grazing by wild animals) occurs naturally. In such neutral communities fine-tuned habitat filtering might be an important process; thus, differences should be sought more in the actual composition of the assemblage.

Some spiders have good dispersal capabilities because of ballooning; therefore, they can track down habitat changes

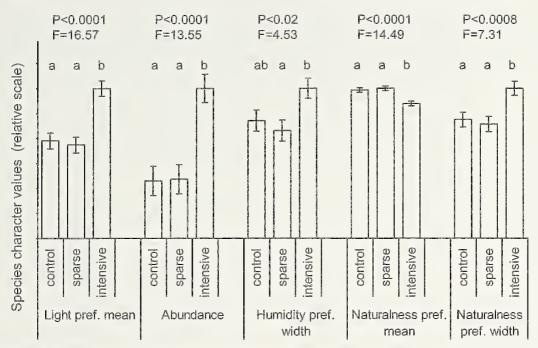


Figure 5.—Species mean character values per pitfall trap (subsample) by grazing areas in the summer samples. One-way ANOVA and Tukey HSD test were performed separately for each character. The effect of year as random factor was originally included, but left out from final models, because in all cases it explained < 1% of variance. Different letters indicate a significant difference by Tukey HSD test at the P = 0.05 level. For plotting, each character was relativized by the maximum mean value.

fairly rapidly. Their assemblages, for instance, can recover rather quickly after a major disturbance like fire (e.g., Spuogis et al. 2005; Samu et al. 2010). Thus, after one year of grazing exclusion we could already expect – and indeed we found – a response from the spider assemblage. However, one of the main findings of the present study was that this qualitative difference (grazing vs. no grazing) was relatively small compared to the quantitative difference we found between the sparsely and intensively grazed areas. Comparing the spider assemblage in the intensively grazed area to the less-

Table 3.—Difference between standardized catches of families in the pitfall trap catches. A family was included in the analysis if more than 30 individuals were caught in total. Mean of percentage differences in catches at sample dates are given between the control + sparse grazing vs. intensively grazed areas, taking the former as the basis. Difference between catches by families was tested with Generalized Linear Mixed Models, after model selection including the fixed effect of 'grazing', 'sampling date' and 'subsample' as random variables and accounting for overdispersion. Poisson error structure and log link function was used. For the effect of intensive grazing vs. control + sparse grazing. z statistics is reported. Note that the Bonferroni-corrected threshold is P = 0.0055.

Family	% difference	z –	P
Dictynidae	-14.3	-0.58	0.560
Gnaphosidae	95.3	1.84	0.066
Hahniidae	-83.6	-7.06	0.0001
Linyphiidae	447.2	4.58	0.0001
Liocranidae	8.8	-1.67	0.095
Lycosidae	37.6	-0.57	0.568
Philodromidae	195.5	0.63	0.529
Salticidae	-78.3	-2.43	0.015
Thomisidae	224.7	3.92	0.0001

grazed or ungrazed areas, we found a striking difference in assemblage structure. By classifying spider species according to their ecological traits, it turned out that less-grazed or ungrazed areas had significantly more species, with a preference for natural habitats. By contrast, in the intensively grazed areas species that also attained high abundances elsewhere in Hungary prevailed, and we could also show that these species generally have wider habitat tolerances (in terms of naturalness and humidity) and have higher preferences for open areas.

We note that trait based approaches – because they are functional - have a much better explanatory power in distinguishing various ecological situations than bulk community measures such as taxon richness. Trait based statistics give more insights into how a community reacts to disturbance (e.g., flooding disturbance: Lambeets et al. 2008; post-fire responses: Langlands et al. 2011). Better insights on changes in assemblage structure are gained by using species' ecological characteristics, even at local scales and with few spatial replicates. The difficulty lies in the availability of good background datasets about specific ecological characteristics. Spiders are good candidates to become a successful indicator group, because databases develop rapidly (Hänggi et al. 1995; Buchar & Růžička 2002; Nentwig et al. 2010). On such bases spiders could reliably indicate conservation value for habitats such as peat bogs (Scott et al. 2006) and grasslands (Samu et al. 2008).

Although species character values gave mean responses broken down by specific ecological traits, family distributions and ISA revealed the families and species that responded to differences in grazing regimes. At a family level, web-building spiders and spider families that typically live on foliage of the grassland vegetation were affected severely by intensive

Table 4.—Results of Indicator Species Analysis comparing the grazing levels 'intensive grazing' vs. 'no or sparse grazing'. IV = Indicator Value, IVRnd = mean of IVs obtained by 4999 random permutations, STD(IVRnd) = standard error of IVRnd, P = probability of obtaining a higher than observed IV in the permutations (all species with P < 0.1 are listed), agrobiont status (constant dominance in arable fields) of species is given according to Samu and Szinetár (2002). Authorities for species names are found in the appendix.

Species	IV	IVRnd	STD(IVRnd)	P	Agrobiont
No or sparse grazing					
Halınia nava	62.3	34.0	5.46	0.0004	
Drassyllus pumilus	44.9	25.1	5.22	0.004	
Alopecosa cuneata	28.8	17.8	4.65	0.0298	
Phrurolithus pullatus	22.4	12.9	4.20	0.0438	
Intensive grazing					
Haplodrassus signifer	52.6	15.7	4.57	0.0002	
Pardosa agrestis	43.5	11.9	3.98	0.0002	yes
Pardosa palustris	34.7	10.5	3.91	0.0002	•
Xerolycosa miniata	35.0	9.4	3.58	0.0002	
Trichoncus affinis	25.0	7.4	2.94	0.0008	
Xysticus kochi	49.5	23.6	5.19	0.0012	yes
Ozyptila scabricula	31.3	12.1	4.15	0.0018	·
Ozyptila claveata	24.5	11.9	4.04	0.0162	
Haplodrassus dalmatensis	15.0	5.1	2.58	0.02	
Drassyllus praeficus	16.9	10.1	3.66	0.046	
Enoplognatha thoracica	10.0	4.3	1.69	0.0796	
Ostearius melanopygius	10.0	4.4	1.74	0.086	
Meioneta rurestris	15.3	9.0	3.67	0.096	yes

grazing, which is in line with changes found in other studies (Churchill & Ludwig 2004; Horvath et al. 2009). Analyzing the species compositions showed that spiders indicate, not only in relative but also in absolute terms, a very good naturalness state of the less-grazed or ungrazed areas. Sparse grazing seems to halt succession at a favorable state, while the disturbance remains minimal for the spider assemblage. During the three years' study, considering the whole pasture, we found many rare and/or specialist species that are representative of good quality dry grasslands. Among these Chalcoscirtus brevicymbialis Wunderlich 1980, new for the Hungarian fauna (Samu & Szinetár 1999), occurs from Germany to Kazakhstan in natural xerothermic rock steppes (Buchar & Růžička 2002; Nentwig et al. 2010). Two other species Panamomops inconspicuus (Miller et Valesova 1964) and Ipa terrenus (L. Koch 1879), also new for Hungary, are mentioned as rare by Buchar and Růžička (2002). Maybe because of the sporadic occurrence of the rarer species, these species could not become significant indicator species of the less-grazed or ungrazed areas. Although many rare species appeared sporadically in our catches, there were statistically more rare species in the less-grazed or ungrazed areas than in the intensively grazed area.

At the intensively grazed area some of the well-known Central European agrobiont species [Pardosa agrestis (Westring 1861), Xysticus kochi Thorell 1872, Meioneta rurestris (C. L. Koch 1836)] were indicators. The indicator status of Ostearius melanopygius (O. P.-Cambridge 1879), a typical cosmopolitan species for disturbed habitats, is also notable, occurring for instance in intensive pastures of New Zealand (Topping & Lövei 1997; Szymkowiak & Woźny 1998).

Both the synthetic measures (richness, abundance) of spider assemblages and concrete species compositions suggested that the sparse grazing area did not differ from the ungrazed area, and it was the intensive grazing that significantly altered the spider assemblage. The neutrality of spider assemblages also emphasized the habitat filtering process; that is, suitability (ecological traits) determined the presence or absence of species. As opposed to no grazing or sparse grazing, intensive grazing opened up spider assemblages for invasion by species with traits that represented various aspects of disturbance tolerance, the appearance of agrobiont species being an example. Complete exclusion of species sensitive to disturbance occurred to a much smaller extent, possibly because of reestablishments from the nearby non-intensively grazed area. Thus, we can conclude that sparse grazing allowed the persistence of rare and otherwise naturalness indicating species, while intensive grazing shifted the species spectrum toward common and disturbance-tolerant species. From a conservation point of view, the utility of grazing depends on its intensity, and it can be either beneficial or adverse for the spider fauna.

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Appendix.—Complete species catches of spiders in the three grazing areas at the Vértesboglár pasture, Hungary.

Speeies	Control	Sparse	Intensive
Igroeca lusatica (L. Koch 1875)	1		
lopecosa accentuata (Latreille 1817)	1	1	
lopecosa cuneata (Clerck 1757)	41	39	2
lopecosa mariae (Dahl 1908)	5	6	4
lopecosa pulverulenta (Clerck 1757)	6	5	1
lopecosa sp.	14	10	2
tella sp.	4	1	-
raeoncus humilis (Blaekwall 1841)	4	•	1
· · · · · · · · · · · · · · · · · · ·		1	1
raneidae sp.	O.	1	3
rgenna patula (Simon 1874)	8	1	3
rgenna sp.	52	7	1
rgenna subnigra (O. PCambridge 1861)	7	11	11
ulonia albimana (Walckenaer 1805)	8	2	4
'ercidia sp.	1	1	
halcoscirtus brevicymbialis Wunderlich 1980	1	3	
halcoscirtus sp.	1	2	
heiracanthium sp.		3	
Tubiona diversa O. PCambridge 1862	3	2	
lubiona sp.	4	1	
· · · · · · · · · · · · · · · · · · ·	1	1	
Coelotes sp.	I	1	
rustulina sp.		1	
Prassyllus praeficus (L. Koch 1866)	1	3	9
Prassyllus pumilus (C. L. Koch 1839)	25	47	4
Prassyllus pusillus (C. L. Koch 1833)	2	1	2
Oysdera erythrina (Walckenaer 1802)		1	
Snoplognatha thoracica (Hahn 1833)			2
Suophrys frontalis (Walckenaer 1802)	7	4	
Juophrys sp.	13	5	
Gnaphosidae sp.	31	28	42
Gongylidiellum murcidum Simon 1884	<i>31</i>	20	1
	156	122	
Iahnia nava (Blackwall 1841)	156	122	9
Jahnia sp.	3	9	
Iaplodrassus dalmatensis (L. Koch 1866)			3
Iaplodrassus signifer (C. L. Koch 1839)	3		27
Iaplodrassus sp.			1
Heliophanus sp.	6	13	2
<i>lypsosinga</i> sp.	5	4	7
pa terrenus (L. Koch 1879)			1
inyphiidae sp.	21	7	10
ycosidae sp.	32	46	16
feioneta rurestris (C. L. Koch 1836)	3	3	5
Aicaria dives (Lucas 1846)	3	4	2
Jemesia pannonica (Herman 1879)	3	1	
Pedothorax apicatus (Blackwall 1850)			1
Stearius melanopygius (O. PCambridge 1879)			2
zyptila claveata (Walckenaer 1837)	4	1	9
zyptila pullata (Thorell 1875)	3	4	3
zyptila scabricula (Westring 1851)	2	5	24
zyptila sp.	1	7	1
achygnatha sp.	1	,	1
anamomops inconspicuus (Miller & Valesova 1964)		2	1
		2	10
ardosa agrestis (Westring 1861)		1	12
ardosa alacris (C. L. Koch 1833)	1	5	1
ardosa bifasciata (C. L. Koch 1834)	3		
ardosa hortensis (Thorell 1872)		1	
ardosa palustris (Linnaeus 1758)	1		45
ardosa prativaga (L. Koch 1870)	1		
ardosa sp.		1	2
hlegra fasciata (Hahn 1826)	2	1	-
hlegra sp.	2	2	
hrurolithus festivus (C. L. Koch 1835)	0		2
	8	8	3
hrurolithus pullatus Kulczynski 1897	18	13	

Appendix—Continued.

Species	Control	Sparse	Intensive
Phrurolithus sp.	3	8	5
Plururolithus szilyi Herman 1879			1
Pisaura mirabilis (Clerck 1757)	2	1	
Robertus arundineti (O. PCambridge 1871)			1
Salticidae sp.	4	2	
Sintula spiniger (Balogh 1935)	1		
Stemonypliantes lineatus (Linnaeus 1758)	1	3	
Synageles sp.		8	
Talavera aequipes (O. PCambridge 1871)	2	5	1
Tapinocyboides pyginaeus (Menge 1869)		1	
Tegenaria sp.			1
Thanatus arenarius L. Koch 1872	4	11	7
Thanatus sp.	8	7	8
Theridiidae sp.	7	5	2
Thomisus onustus Walckenaer 1806	1	1	
Tibellus sp.	1	1	
Trachyzelotes pedestris (C. L. Koch 1837)	4		1
Trichoncus affinis Kulczynski 1894			15
Trichopterna cito (O. PCambridge 1872)			1
Trochosa robusta (Simon 1876)			1
Trochosa terricola Thorell 1856	1		
Urocoras longispinus (Kulczynski 1897)	4	2	3
Xerolycosa miniata (C. L. Koch 1834)			15
Xysticus acerbus Thorell 1872	1		
Xysticus audax (Schrank 1803)		1	
Xysticus cristatus (Clerck 1757)			1
Xysticus koclii Thorell 1872	4	10	27
Xysticus sp.	1	1	4
Xysticus striatipes L. Koch 1870	4	15	
Zelotes electus (C. L. Koch 1839)	13	5	13
Zelotes gracilis Canestrini 1868	37	35	32
Zelotes longipes (L. Koch 1866)	25	16	15
Zodarion rubidum Simon 1914			1
Zora parallela Simon 1878	2		
Zora sp.	3		
Number of individuals	649	584	431
Number of species	67	67	59

Molecular characterization of Russian wheat aphid consumption by spiders in winter wheat

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Abstract. Accurate characterization of predator-prey linkages in agroecosystems is important prior to the implementation of conservation biological programs. The Russian wheat aphid, *Diuraphis noxia* (Hemiptera: Aphididae), is a significant pest of wheat and barley in the United States. This research utilized molecular gut-content analysis as a minimally disruptive technique to characterize the trophic connectivity between two spider species, *Tetragnatha laboriosa* Hentz 1850 and *Pardosa sternalis* (Thorell 1877), and *D. noxia*. We describe the development of species-specific primers that amplify a 227 bp fragment of *D. noxia* COI mtDNA to identify the frequency of predation under varying aphid densities and developmental stages of winter wheat. We tested the hypotheses that predation rates on *D. noxia* would be highest for both spider species at the greatest aphid infestation level in the aphid-resistant wheat cultivar plots and that densities of *T. laboriosa* would be highest at the highest aphid infestation level in the aphid-resistant cultivars. Despite short detection periods of prey DNA in the laboratory, 32% and 48% of field-collected *T. laboriosa* and *P. sternalis* spiders screened positive for *D. noxia* DNA, respectively. *T. laboriosa* densities were highest at the highest aphid infestation level. Aphid-resistant wheat cultivars did not impact predation rates or densities. Additionally, *P. sternalis* predation on *D. noxia* increased with increasing aphid infestation levels. Given the high predation rates on *D. noxia* and their association with increased aphid densities, both spider species represent important natural enemies within wheat agroecosystems, and further research is required to quantify their impact on aphid populations.

Keywords: Diuraphis noxia, biological control, predator-prey interactions, molecular gut-content analysis, generalist predators

Given the premise that natural enemies, with diverse modes of foraging and asynchronous life cycles, act as a whole to limit pest populations as opposed to individual species acting alone (Sunderland et al. 1997), it is essential to understand the behavior of all constituent parts of the community. Importantly, many are often present before pests arrive and thereby impact pests during colonization (Edwards et al. 1979; Chiverton 1987; Landis & van der Werf 1997; Harwood et al. 2004). Given this attribute, characterizing their feeding behavior in agroecosystems forms an important component of developing pest management approaches. However, understanding the trophic interactions between predators and prey can be complex. Observations of predator-prey interactions are often disruptive to the study system and inherently biased in terms of what can be "observed" and the time of sampling. In the last 20 years, molecular techniques alleviated many of these concerns and have contributed to the understanding of trophic relationships in the field (reviewed by Symondson et al. 2002; Sheppard & Harwood 2005; Weber & Lundgren 2009).

The Russian wheat aphid, *Diuraphis noxia* Kurdjumov 1913 (Hemiptera: Aphididae), is a pest of wheat, *Triticum aestivum* L. (Poales: Poaceae), and other small grains in all wheat-growing countries except Australia (Elliott et al. 1998). Aphid-resistant cultivars that prevent the wheat leaf from curling are now widely planted, which will likely expose aphids on the plant surface (Hawley et al. 2003). Thus, external disturbances like wind, rain, and predators (von Berg et al. 2008) could trigger a higher falling rate than susceptible cultivars, thereby increasing contact with epigeal predators. However, the use of resistant cultivars has been compromised by the introduction of Russian wheat aphid biotype 2 (Haley et al. 2004), creating a further need to consider

epigeal predators for pest management. Spiders are a major component of this fauna (Sunderland & Greenstone 1999), aggregate to areas of high prey density (Harwood et al. 2001, 2003), and feed on a variety of crop pests, including aphids (e.g., Chiverton 1987; Sunderland et al. 1987; Winder et al. 1994; Harwood et al. 2004, 2005; Oelbermann & Scheu 2009). Additionally, the high falling rates of aphids from wheat plants (Kerzicnik et al. 2010) suggests that if predator densities are sufficiently high, both epigeal and web-building spiders could exert some degree of control. Identifying the foraging behavior of such species is therefore required in order to determine the potential roles of these species for biological control.

Tetragnatha laboriosa Hentz 1850 (Araneae: Tetragnathidae) is a dominant predator within several agroecosystems (Young & Edwards 1990; Nyffeler & Sterling 1994) and can rapidly recolonize following disturbance (Howell & Pienkowski 1971) by means of ballooning throughout their lifetime (Bell et al. 2005). It builds small webs, capturing many aphids (Culin & Yeargan 1982; Nyffeler & Sterling 1994; Jmhasly & Nentwig 1995) and small flies (Provencher & Coderre 1987). Spiders in the genus Pardosa (Araneae: Lycosidae) are also commonly found in agroecosystems (Marshall & Rypstra 1999; Samu & Szinetár 2002; Öberg & Ekbom 2006), and Pardosa sternalis (Thorell 1877) is particularly common in northern Colorado and, as with most epigeal predators, Pardosa are affected by plowing, tillage and mechanical weed control (Thorbek & Bilde 2004). They are active prey hunters, have a broad feeding niche (Bailey & Chada 1968) and impact pest species at a different stratum in the crop.

Since *T. laboriosa* is a known aphid predator and both spider species are dominant in Colorado wheat, it would be

expected that these two spider species feed on *D. noxia* in the field. For this study, the following hypotheses were examined:

1) *T. laboriosa* densities will be highest at the highest aphid infestation level in aphid-resistant wheat cultivar plots and 2) *T. laboriosa* and *P. sternalis* predation on aphids will be highest at the highest aphid infestation level in aphid-resistant wheat cultivar plots. Using PCR with species-specific primers, the goal of this study was to measure the frequency with which *T. laboriosa* and *P. sternalis* prey on *D. noxia* in a winter wheat agroecosystem in order to identify their potential role in biological control.

METHODS

Study Site and Planting Regime.—Research was conducted in winter wheat at the Colorado State University Agricultural, Research, Development and Education Center (ARDEC), Fort Collins, Colorado, USA, (GPS coordinates: 40.65099°N, 104.99671°W; elevation 1534 m). The site was irrigated once prior to planting on 3 September 2007 to ensure uniform plant emergence, and wheat was grown according to standard agronomic practices for the region. The wheat (eultivar STARS 02RWA2414-11/5*CO00554) was planted on 11 September 2007, and sampling occurred during the 2008 growing season. No herbicides were applied during the experiment.

Experimental Design.—This study was a split-plot design with repeated measures. The whole-plot factor replicated eight times was aphid infestation level, and the split-plot factor was the infestation level of aphids in wheat cultivars. Split plots were 3.24 m² with six wheat rows, and "Hatcher" wheat was planted as a buffer between and outside of the plots. There were three aphid infestation levels $(0 \times, 1 \times, \text{ and } 10 \times)$ to examine predation under varying aphid densities. Within each plot, winter wheat plants were infested with greenhouse-reared (L16:D8 cycle, 24 °C, 65% humidity) D. noxia biotype 2 using a Davis inoculator (Davis & Oswalt 1979). Four, 1-m rows in the center of the $1\times$ and $10\times$ plots were infested with approximately 246 and 2,460 biotype RWA2 aphids, respectively, on 7 March 2008. No aphids were added to the $0\times$ infestation level plots. Infestation numbers to be applied in the field were estimated by using the Davis inoculator to deliver aphids to 10 Petri dishes. The number of D. noxia per inoculator delivery per Petri dish was averaged, providing an estimate of the number of aphids delivered to wheat in the field. Two different wheat cultivars were used, one resistant and one susceptible to *D. noxia*.

Spider Field Collection.—Spiders were hand-collected and numbers counted from the entire area of each plot twice weekly between May–July 2008. The density of spiders was low, so they were pooled into five wheat stages: Zadoks 40, 50, 60, 70, and 80 (Zadoks et al. 1974). Tetraguatha laboriosa was sampled between 07:30–09:00, when dew allowed for easy web detection and at a time that corresponds to increased feeding (Culin & Yeargan 1982). Pardosa sternalis was sampled between the hours 07:30–9:00 or 13:00–15:00. Individual spiders were transferred into 1.5 mL microcentrifuge tubes filled with chilled 100% ethanol and transferred to the laboratory in a cooler (\leq 4 °C). Tetragnatha laboriosa females were difficult to identify without epigynal dissections, which could contaminate the specimens. Thus, they were grouped into an "immature/female" category. Tetragnatha laboriosa

was not recorded after 19 June 2008, by which time *P. sternalis* was scarce, so the collection of both spider species was discontinued after this date.

Aphid Sampling.—The mean density of *D. noxia* on wheat tillers was estimated by removing all wheat tillers from a random 14 cm^2 area between two of the four aphid-infested rows every two weeks from each replicate of the $0\times$, $1\times$ and $10\times$ plots, for a total of five dates. Tillers were cut and removed at ground level, placed into a 3.8 L plastic bag, and held on ice until they were transferred into Berlese funnels for 24 h (Tragardh 1933). Aphids were subsequently extracted into 75% ethanol for long-term storage and eounting.

Spider Feeding Experiment.—A laboratory feeding study was performed to validate the detectability of D. noxia DNA within spiders following consumption. Pardosa sternalis were collected alive from dry pitfall traps set in winter wheat adjacent to the aforementioned plots. Tetragnatha laboriosa spiders were collected with aspirators from the same adjacent wheat areas. Spiders were maintained in 100 × 15 mm Petri dishes with a moist Plaster of Paris substrate for water supply on a L16:D8 cycle with fluctuating day (24 °C) and night (20 °C) temperatures (Lab-Line Biotronette Plant Growth Chamber, Lab-Line Instrument, Inc., Melrose Park, Illinois, USA), conditions comparable to those observed in the field. Moisture was provided by spraying the inside of each dish twice daily with water. Spiders were fed two to three Drosophila melanogaster Meigen 1830 (Diptera: Drosophilidae) every other day for approximately two weeks to reduce stress and maintain the health of the spider prior to the start of the experiment. Spiders were starved for approximately 7 d, and then fed one D. uoxia biotype 2 aphid. The spiders were individually observed to feed and were stored at -20 °C in 100% ethanol at the following post-feeding times (in h): 0 (i.e., immediately after feeding), 4, 8, 12, 16, and 24, with eight individuals represented for each time period. Spiders were maintained in the plant growth chamber during their digestion period before freezing. Eight starved spiders of each species served as negative controls.

Primer Design.—An 1146 bp sequence of the mitochondrial cytochrome c oxidase subunit I (COI) gene from D. noxia was retrieved from the GenBank database (Accession #FJ232620). This, sequences from P. sternalis and T. laboriosa (sequenced from the universal primers C1-J-1718 and C1-N-2191 (Simon et al. 1994)), and those of the following aphid species derived from GenBank: Diuraphis frequens (Walker 1848) (Accession #FJ232622), D. tritici (Gillette 1911) (Accession #FJ232621), Rhopalosiphum padi (Linnaeus 1758) (Hemiptera, Aphididae) (Accession #AY594671) and R. maidis (Fitch 1856) (Accession #AY594673) were aligned using ClustalW (Larkin et al. 2007) within the BioEdit sequence alignment editor (Version 7.0.5, Tom Hall, Ibis Therapeutics, Carlsbad, California, USA). A pair of primers (RWACOIF-F: 5'-CACTTATTA-TGTAGTAGCACATTTTCAT-3'; RWACOIR-R: 5'-TTA-GGATAATCTGTATATCGTCGTGGT-3') amplifying a 227 bp sequence, were designed using Primer 3 software (Version 2.2.3, S. Rozen & H. Skaletsky, Whitehead Institute, Cambridge, Massachusetts, USA and Howard Hughes Medical Institute, Chevy Chase, Maryland, USA), analyzed with Oligo Analyzer (Version 3.1, Integrated DNA Technologies, Inc., Coralville, Iowa, USA), and optimized by performing a

Table 1.—Arthropods tested with *Dinraphis noxia* primer pairs.

Order	Family	Species
Acari	Tetranychidae	Oligonychus pratensis (Banks 1912), Petrobia latens (Müller 1776)
Araneae	Gnaphosidae	Drassyllns nannellns Chamberlin &Gertsch 1940
	Lycosidae	Schizocosa mccooki (Montgomery 1904)
	Thomisidae	Xysticus pellax O.PCambridge 1894
Coleoptera	Carabidae	Bembidion quadramaculatum (Linnaeus 1761), Poecilus sp.
•	Coccinellidae	Coccinella septempunctata Linnaeus 1758, Hippodamia convergens Guérin-Méneville 1842,
		Hippodamia parenthesis Say 1824, Coccinella transversognitata Faldermann 1835, Scynmus sp.
Collembola	Isotomidae	Undetermined sp.
Diptera	Culicidae	Culex pipiens L. 1758, Culex tarsalis Coquillett 1758
•	Taehinidae	Phasia sp.
Hemiptera	Anthocoridae	Orius sp.
	Lygaeidae	Nysins cf. raphanus Howard 1872
	Miridae	Lygus sp.
	Nabidae	Undetermined sp.
	Pentatomidae	Undetermined sp.
	Rhopalidae	Arhyssus lateralīs (Say 1825)
Homoptera	Aphididae	Acyrthosiphon pisum Harris 1776, Dinraphis frequens, Dinraphis tritici, Rhopalosiphum padi,
		Rhopalosiphum maidis, Schizaphis graminum (Rondani 1852), Sitobion avenae (Fabricius 1775).
		Sipha elegans del Guercio 1905
Thysanoptera	Thripidae	Anaphothrips obscurns (Muller 1776)

gradient PCR and by adjusting reagent concentrations, number of cycles, and the denaturation, annealing, and extension times.

DNA Extraction.—The whole-body extraction of DNA from spiders was performed with Qiagen DNeasy Animal Tissue kits (Qiagen, Valencia, California, USA) following the manufacturer's animal tissue protocol. The DNA concentration from the extractions was quantified with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA), using 1 μ L of template. The ratio of sample absorbance at 260 and 280 nm was used to assess the purity of the DNA. DNA concentrations from the spiders ranged from 50–450 ng/ μ L. DNA concentrations from single aphids ranged from 1–6 ng/ μ L. After measurement, total spider DNA extractions were diluted to 50 ng/ μ L for standardization and stored at -20 °C.

PCR amplification and purification.—PCR reactions using the RWA-specific primers (25 µL) included the following reagents: 2.5 µL of Takara 10× Buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM MgCl₂), 1.0 μL of each primer (0.4 µM), 2 µL of Takara dNTP mixture (dATP, dCTP, dGTP, dTTP, 2.5 mM of each), 0.625 units/µL of Takara Taq HS DNA polymerase (0.125 μ L), and 5 μ L of template DNA. The PCR protocol included the following: an initial denaturation step of 3 min at 94 °C; followed by 35 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 60 °C, and extension for 60 s at 72 °C; and a final extension step of 72 °C for 5 min. PCR products were separated by electrophoresis in 2% agarose gels (Fisher Scientific, BP160-500, Pittsburgh, Pennsylvania, USA), post-stained with ethidium bromide for 1 h, and photographed under UV light (Syngene Blue Light Transilluminator, Syngene, Frederick, Maryland, USA). Positive and negative D. noxia controls were included in each PCR experiment.

The PCR product from one *D. noxia* (positive control) was purified with a Mo Bio Ultraelean Purification Kit (MO BIO Laboratories, Inc., Carlsbad, California, USA) following the

manufacturer's protocol and sequenced following the dideoxychain-termination method at the University of Washington's High-Throughput Sequencing Solutions. The nucleotide identity for both primer pairs matched 100% with *D. noxia*, indicating that the correct region was amplified for PCR.

Cross-reactivity testing.—Extensive primer specificity testing was conducted to eliminate occurrence of false positives due to the cross-reactivity of primers. Non-target prey (one individual each) were collected from the wheat field where sampling occurred and also from other laboratories and placed in 100% ethanol (Table 1). DNA from these individuals was extracted as described above, and PCRs were conducted with the *D. noxia*-specific primers to ensure that the DNA of these non-target species did not amplify with these primers. The non-target prey were not amplified by the selected primers.

Statistical Analyses.—Molecular half-lives, i.e., the time at which half of the predators are positively identified with prey DNA following consumption (after Greenstone & Hunt 1993), were calculated for each species using the "probit" procedure in SAS (SAS Institute 2002–2008). Frequency tabulations were performed using the "PROC FREQ" procedure in SAS (SAS Institute 2002-2008) with the Chi-Square statistic and associated probabilities to determine whether the percentage of field spiders positive for the presence of D. noxia DNA was associated with aphid infestation level, aphid-resistant eultivars or wheat stage. Data from sampled plots were analyzed for T. laboriosa for the effects of wheat stage, infestation level, and level of resistance using the "Mixed" procedure in SAS (SAS Institute 2002–2008) with the REML estimation method and the Kenward-Roger approximation for degrees of freedom (Kenward & Roger 1997). Repeated measures models with autoregressive errors and unequal variances across dates were evaluated and used when justified by Akaike's Information Criterion (Burnham & Anderson 2002), which are used to measure the quality of fit. Density analyses were performed for Pardosa sternalis, but only densities during wheat stages

Wheat Stage _		Infestation level	
(Zadoks)	0×	1×	10×
40	0.00 Aa	0.00 Ba	0.00 Ba
50	0.00 Aa	0.06 Ba	0.06 Ba
60	0.13 Ab	1.19 Ab	1.69 Aa
70	0.06 Aa	0.31 Ba	0.13 Ba
80	0.00 Aa	0.00 Ba	0.00 Ba

were used for analyses due to its high mobility and varied collection times. T. laboriosa densities were square-root transformed (x + 0.5) for the density analyses and pooled into the following five wheat stages: Zadoks 40, 50, 60, 70, and 80. When significant effects were observed ($P \le 0.05$), Tukeyadjusted pairwise comparisons were performed. Raw means are given in the tables and figures presented herein.

For *D. noxia* densities, mixed models with autoregressive errors and unequal variances across dates were eonsidered. Aphid densities were log transformed [log₁₀ (x)]. A model was selected based on the lowest AIC value, and restricted maximum likelihood (REML) was used as a method for estimating the parameters of the model (SAS Institute 2002–2008). A mixed model with an autoregressive order 1 covariance structure with heterogeneous variances across dates (ARH(1)) was ehosen as the appropriate model.

RESULTS

Field collected spiders.—Tetragnatha laboriosa: Sixty-four T. laboriosa were eollected in 2008. Of these, 3% were male, 53% were immature (22% penultimate males), and 44% were either female or immature. Tetragnathids appeared at Zadoks 50, peaked at Zadoks 60, and declined at Zadoks 70 and 80. The immatures were assumed to be T. laboriosa, as no other Tetragnatha species were present at this site (L.M. Kerzicnik, unpublished data). Tetragnatlia laboriosa densities were significantly higher at Zadoks 60 than any other wheat stage $(F_{4,217} = 43.70, P < 0.0001)$ and subsequently declined after this stage. For infestation level, T. laboriosa densities differed $(F_{2,217} = 12.08, P < 0.0001)$ and were higher in the 1× $(t_{217} =$ -3.93, P = 0.0001) and $10 \times (t_{217} = -4.52, P < 0.0001)$ infestation levels compared to the 0× infestation level. Similarly, T. laboriosa densities were affected by wheat stage and infestation level combined ($F_{8, 217} = 8.90, P < 0.0001$). The highest mean spider density occurred during Zadoks 60 at the 10× aphid infestation level, and densities were higher at the 10× infestation level compared with the 1× (t_{217} = 2.99, P = 0.0031) and $0 \times (t_{217} = -9.33, P < 0.0001)$ infestation levels at this stage. No significant differences among wheat stages were found at the $0\times$ infestation level (Table 2), and wheat resistance did not affect densities of T. laboriosa $(F_{1, 211} =$ 1.67, P = 0.1974).

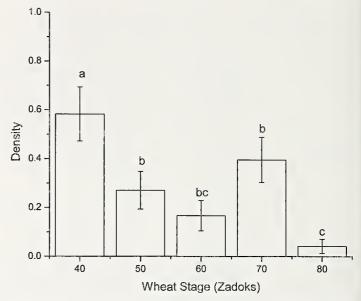


Figure 1.—Mean density (± SE) of *Pardosa sternalis* per wheat stage (Zadoks), averaged over wheat varieties and infestation levels, Fort Collins, Colorado, 2008. Columns marked by the same lower case letters are not significantly different.

Pardosa sternalis: Seventy-one P. sternalis were collected in 2008. Of these, 28% were male, 51% were immature, and 21% were female. The immatures collected were assumed to be P. sternalis, as no other Pardosa were present at the field site (L.M. Kerzicnik, unpublished data). Wheat stage affected the number of P. sternalis collected ($F_{4, 277} = 7.43$, P < 0.0001) with mean densities highest at Zadoks 40 (Fig. 1).

Aphids.—Aphid densities varied with the level of resistance $(F_{I, 5l.6} = 79.99, P < 0.0001)$, with susceptible treatments supporting considerably more aphids than the resistant treatments (Table 3), although there was also an interaction between date and resistance ($F_{4,70.1} = 5.74$, P = 0.0005). Aphid densities also differed between infestation levels ($F_{2,18.9}$ = 595.96, P < 0.0001), with the highest aphid densities at the $10\times$ infestation level and densities within the $0\times$ treatments remained close to, or at, zero throughout the experiment. The density of aphids varied with sample date ($F_{4,71} = 208.78, P <$ 0.0001), with densities peaking on June 4 and then declining in all treatments subsequently (Table 3). There was an interaction between date and infestation level for aphid density (F_8) $_{86.8} = 37.84$, P < 0.0001). The $10 \times$ infestation level was higher than the 1× level, averaged over the level of resistance on May $4 (t_{32.21} = -6.36, P < 0.0001)$, May 21 $(t_{34.5} = -5.74, P <$ 0.0001), and June 4 ($t_{41.8} = -3.74$, P < 0.0001). On May 21, June 4, and June 18 there was a difference between aphid densities in the resistant and susceptible treatments, averaged over infestation levels, respectively ($t_{27.2} = -4.76$, P <0.0001), $(t_{38.5} = -4.62, P < 0.0001)$, and $(t_{31.8} = -6.59, P$ < 0.0001). An interaction between wheat stage by resistance by infestation level was observed for aphid density ($F_{8;85.9}$ = 4.04, P = 0.0014) (Table 3). The highest aphid densities occurred at the 10× susceptible treatment at Zadoks 50, and densities within the 0× resistant and susceptible treatments remained close to, or at, zero throughout the experiment.

Spider feeding experiment.—Results of the spider feeding experiment showed that 100% of *T. laboriosa* tested positive

Table 3.—Mean density of *Diuraphis noxia* on wheat tillers (cm $^{-2}$ d $^{-1}$) at the 1× and 10× infestation levels for resistant (R) and susceptible (S) wheat lines in Fort Collins, Colorado, 2008. Means within a column followed by the same capital letters are not significantly different and represent differences between dates at each treatment. Means within rows followed by the same lower case letters are not significantly different and represent differences between treatments at each date and wheat stage.

	Wheat Stage	07	00	1		107	105
Date	(Zadoks)	0×R	0×S	$1\times R$	1×S	10×R	10×S
4 May	30	0.00Bc	0.00Cc	0.19Cb	0.38Cb	0.86Ca	1.16Ca
21 May	40	0.00Bd	0.01Cd	1.89Be	3.94Bb	5.04Bb	12.27Ba
4 June	50	0.10Ad	0.22Bd	8.35Ac	16.86Ab	17.64Ab	59.74Aa
18 June	70	0.01Bc	0.49Ac	7.49Ab	14.05Aa	6.35Bb	19.87 B a
2 July	80	0.02Bb	0.20Ba	0.19Ca	0.23Ca	0.20Ca	0.22Da

for *D. noxia* immediately after feeding and declined thereafter such that no predators were positive after 12 h. The molecular half-life was calculated as 4.2 ± 1.1 (SD) h. Similarly, 100% of *P. sternalis* tested positive for *D. noxia* DNA immediately after feeding but detection declined rapidly thereafter, indicating rapid degradation of this primer region in these predators. The molecular half-life for *D. noxia* DNA in *P. sternalis* was calculated as 2.0 ± 0.4 (SD) hrs. Starved predators always screened negative.

Predation and Gut-Content Analysis.—*Tetragnatha laboriosa:* Of the 64 *T. laboriosa* collected from all wheat stages and infestation levels, 32.8% were positive for the presence of *D. noxia.* The number of spiders testing positive for the presence of *D. noxia* DNA was not significantly related to wheat stage $(X^2)_3 = 3.18$, P = 0.37, infestation level $(X^2)_2 = 1.61$, P = 0.45, or between aphid-resistant cultivars $(X^2)_1 = 0.22$, P = 0.64.

Pardosa sternalis: Of the 71 P. sternalis collected, 48% were positive for the presence of D. noxia DNA. The number of spiders testing positive for the presence of D. noxia DNA was not significantly different between wheat stages ($X^2_4 = 6.08$, P = 0.19) or resistance levels ($X^2_1 = 0.07$, P = 0.79). However, as aphid infestation level increased from $0 \times$ to $10 \times$, the percentage of spiders testing positive for D. noxia increased significantly ($X^2_1 = 8.91$, P = 0.0028) (Fig. 2).

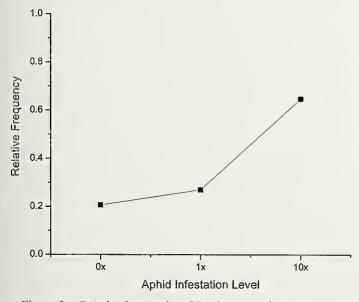


Figure 2.—Relative frequencies of *Pardosa sternalis*, averaged over wheat varieties and wheat stages, testing positive for the presence of *Diuraphis noxia* DNA, Fort Collins, Colorado, 2008.

DISCUSSION

Conservation biological control requires a fundamental understanding of the role natural enemies play in agroecosystems and the temporal dynamics of their populations. This study examined the relationship between two abundant predators, *T. laboriosa* and *P. sternalis*, and a major pest of winter wheat, *D. noxia*, by testing the hypothesis that both spider density and predation would increase with increasing aphid infestation level within resistant cultivars. Most importantly, both predators frequently screened positive for the presence of *D. uoxia* DNA (33% and 48%, respectively), indicating that significant levels of pest consumption occurred under natural field conditions.

Over 92% of T. laboriosa were collected at the $1 \times$ or $10 \times$ aphid infestation levels, and the majority were present within the 10× infestation level, demonstrating some level of aggregation to D. noxia. Also, the abundance of T. laboriosa peaked at Zadoks 60, the growth stage that occurred between the two highest D. noxia densities, and was highest once again within the 10× aphid infestation level. Tetragnatha laboriosa also showed evidence for residing in, and constructing webs where, aphid densities were highest, a phenomenon also reported as occurring with linyphiid spiders in alfalfa (Romero & Harwood 2010). This spider balloons throughout its lifetime (Bell et al. 2005), which is an important trait to possess when residing within agroecosystems that are characterized by frequent disturbances. Indeed, the rapid recolonization of highly disturbed agricultural habitats is critically important in biological control (Welch et al. 2011), and these combined attributes are optimal for effective biological control and further indicate an association with the pest.

Pardosa sternalis densities were highest at Zadoks 40 and Zadoks 70, and consumption of D. noxia was also highest at these times. Interestingly, a concurrent study within the same treatments found that the falling rate of D. noxia was significantly higher in the resistant cultivars compared with its susceptible counterparts at the $10\times$ aphid infestation level at both of these wheat stages (Kerzicnik et al. 2010). Thus, P. sternalis is likely utilizing the aphid prey source when it is available.

Biological control is most efficient when generalist predators are present in the crop early before pests reach peak densities (Edwards et al. 1979; Ekbom & Wiktelius 1985; Chiverton 1986; Birkhofer et al. 2008). *Pardosa sternalis* was most abundant at Zadoks 40, prior to peak aphid densities, and demonstrated a high aphid consumption rate, traits characteristic of successful biological control agents. Additionally, the

cursorial spiders *Xysticus cristatus* (Clerck 1757) (Araneae: Thomisidae) and *Pardosa palustris* (Linnaeus 1758) (Araneae: Lycosidac) suppressed growth of the English grain aphid, *Sitobion avenae* (Fabricius 1775) (Homoptera: Aphididae) (Birkhofer et al. 2008). This contrasts with *T. laboriosa*, which colonized wheat fields at peak aphid densities and dispersed to an adjacent corn crop as *D. uoxia* densities declined by over 97% from Zadoks 70 to Zadoks 80 (L.M. Kerzienik, pers. obs.). Therefore, it is important to understand agricultural management practices that encourage the early colonization of generalist predators such as *P. sternalis* to prey on pests at lower densities.

The retention time of target DNA for both T. laboriosa and P. sternalis was particularly short (4.2 h and 2.0 h, respectively). This contrasts to a number of other studies; for example, collembola DNA has been detected from within linyphiid (Araneae: Linyphiidae) spider guts for up to 24 h post feeding (Agustí et al. 2003). Short retention times, however, are not always disadvantageous when studying predator-prey interactions (Sheppard & Harwood 2005). A shorter retention time can provide detailed information about a recent predation event, and in a study tracking the predation of Rhopalosiphum padi by Pardosa, the molecular half-life was only 3.7 h (Kuusk et al. 2008). Although physiological and morphological differences could also play a part, predator to prey size ratio can be important for detection of prey material. Using monoclonal antibodies and ELISA, pink bollworm eggs, Pectinophora gossypiella (Saunders 1844) (Lepidoptera: Gelechiidae), were detected longer from inside the guts of a minute pirate bug, Orius insidiosus (Say 1832) (Hemiptera: Anthocoridae), than a coccinellid beetle, Hippodamia convergens Guérin-Méneville 1842 (Coleoptera: Coccinellidae) (Hagler & Naranjo 1997). Both P. sternalis and T. laboriosa were approximately five times larger than a single D. uoxia prey. Other studies that indicate longer retention times with prey DNA in feeding trials were more representative of a smaller predator: prey size ratio (Agustí et al. 2003; Monzó et al. 2010). Nevertheless, predation rates were high for both species in the field, validating the approach used here and suggesting very high levels of pest consumption by these spiders during diurnal hours. Aphid prey were also more readily available at this time, and other studies in alfalfa indicated significantly higher prey availability for linyphiid spiders at night (Romero & Harwood 2010).

Although spiders are unlikely, alone, to reduce pest densities below economically damaging levels, syncrgism could take place with multiple predators with divergent foraging strategies to allow for increased predation (Losey & Denno 1998; Schmidt et al. 2003). Given the high rates of predation on *D. uoxia* for both spiders, it is likely that *T. laboriosa* can capture many aphids within the above-ground wheat while *P. sternalis* can intercept aphids on the ground prior to re-colonization of the crop, identifying an important predatory component for pest management.

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A new phylogeny of *Anelosimus* and the placement and behavior of *Anelosimus vierae* n. sp. from Uruguay (Araneae: Theridiidae)

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Abstract. Available evidence suggests that sociality in the spider genus *Anelosimus* Simon has evolved as a gradual transition from short-term maternal care to permanent sociality. The discovery and description of new species displaying various intermediate levels of sociality deepens our understanding of this transition. Here I use five molecular loci (28S, ITS2, COI, 16S, ND1) to phylogenetically place specimens of an intermediate-social population from Uruguay, previously and tentatively identified as the widespread and common *A. studiosus* (Hentz 1850). The Chinese *A. chonganicus* Zhu 1998 is also phylogenetically placed for the first time, and new sequences from four additional *Anelosimus* species and two theridiid genera (*Audifia* Keyserling, *Tekellina* Levi) are all combined with previously published data to reconstruct a novel phylogeny of *Anelosimus* spiders. This phylogeny recaptures previously well-established groups and reiterates well-known themes such as the multiple origin of sociality. The Uruguayan specimens nest outside of *A. studiosus*, and I therefore describe these as a new species, *Anelosimus vierae* sp. n. and summarize existing data on its behavior in the context of social evolution. I also synonymize *A. tunguralua* Agnarsson 2006 with *A. studiosus* new synonymy. Finally, I define the subfamily Anelosiminae, containing *Anelosimus* and *Kocliura* Archer; Anelosiminae is sister to the diverse Theridiinae.

Keywords: Cobweb spiders, intermediate social, social evolution, subsocial

Only a few of the over 41,000 described spiders (Platnick 2010) are social, and most of them occur in a few clusters of phylogenetically closely-related species (Avilés 1997; Agnarsson et al. 2006a; Avilés et al. 2006; Lubin & Bilde 2007; Johannesen et al. 2007, 2009). The cobweb spider genus Anelosinns Simon 1891, for example, contains the majority of all cooperative spiders, and recent work has uncovered many new Anelosiums species (Agnarsson 2005, 2006; Agnarsson & Kuntner 2005; Agnarsson & Zhang 2006; Agnarsson et al. 2010). Anelosiums species display a range of social behavior from solitary with short-term maternal care (Agnarsson et al. 2006b) to permanent, highly social behavior (Vollrath 1986; Avilés 1997; Avilés et al. 2001). The majority of Anelosimus species are subsocial with single-female nests, involving sibling cooperation until adulthood, followed by dispersal and outbreeding, and equal sex ratios (Avilés 1997). Eight species are permanently social with multi-female nests showing adult cooperation and successive generations remaining in the natal nests, with inbreeding and interdemic selection resulting in strongly female-biased sex ratios (Avilés 1993, 1997; Avilés et al. 2007). The phylogenetic relationships among these species suggest multiple, gradual, transformations from subsocial to permanently social (Agnarsson et al. 2006a, 2007a), as also seen in the distantly related eresid spiders, genus Stegodyphus Simon 1873 (Bilde et al. 2005; Johannesen et al. 2007, 2009). This transition presumably occurs via the various intermediate social stages (occurrence of multi-female nests, partial outbreeding, intermediate sex ratio bias) (Powers & Avilés 2003; Avilés & Bukowski 2006) that are displayed by a few existing species, such as A. jabaquara Levi 1956, A. dubiosus (Keyserling 1891) and certain populations of A. studiosus (Hentz 1850) (Marques et al. 1998; Vasconcelos-Netto & Mello 1998; Jones & Parker 2000, 2002; Gonzaga & Vasconcellos-Neto 2001, 2002; Jones et al. 2007). Thus the discovery and phylogenetic placement of further species with intermediate social structures will deepen the understanding of social evolution in spiders.

I recently revised the American Anelosimus species (Agnarsson 2005, 2006) based on an examination of material from all major museums worldwide containing American material. Concurrently, behavioral data were being collected for some of the potentially new species (by L. Avilés and coworkers) which greatly helped species delimitation. Nevertheless, I concluded that these revisions were incomplete, not only because future sampling would likely uncover new species, but also because, in some cases, examination of morphology alone seemed insufficient to adequately delimit species from existing material (Agnarsson 2006). The first molecular phylogeny (Agnarsson et al. 2007a) showed good congruence with morphological taxonomy in general, especially for taxonomic decisions that were based on morphological and behavioral data combined, but this study also pointed to some potential problems. For example, A. tungurahua Agnarsson 2006 had seemed subtly distinct from A. studiosus morphologically, but nested within A. studiosus in gene trees of multiple loci (Agnarsson et al. 2007a). Further, a specimen from Uruguay tentatively identified as 'A. studiosus' did not group close to A. studiosus in preliminary analyses (I. Agnarsson unpublished data). Now, several studies on the behavior of this Uruguayan population have been conducted, demonstrating some differences from the behavior of A. studiosus, and have highlighted that this population shows an intermediate social structure (Albo et al. 2007; Viera et al. 2006, 2007a, b, c; Viera & Albo 2008). For example, in the Uruguayan population multi-female nests are not uneommon, and primary sex ratios are female-biased 2:1 (Viera et al. 2007a).

Here, I add three specimens of the Uruguayan population and additional sequences from five other *Anelosimus* species (*A. analyticus* (Chamberlin 1924), *A. chonganicus* Zhu 1998, *A. ethicus* (Keyserling 1884), *A. octavius* (Agnarsson 2006), *A. rnpmmni* Levi 1956), and two new outgroups (*Audifia* Keyserling 1884 and *Tekellina* Levi 1957) to the molecular phylogenetic analyses of Agnarsson et al. (2007a, 2010) and, following the phylogenetic results, describe the Uruguayan

population as a new species. Finally, I summarize what is known about its behavior, in the context of social evolution.

METHODS

Phylogenetics.—Specimens were collected in the field (from Montevideo, Uruguay, 34°53′15"S, 56°08′33"W) by C. Viera and collaborators, and fixed in 95% ethanol. I obtained sequences of mitochondrial (16S, ND1, COI) and nuclear (28S, ITS2) loci from three individuals of A. vierae, using primers and settings as described in Agnarsson et al. (2007a) and Agnarsson (2010). I also obtained for the first time sequences from three specimens of A. chonganicus and additional sequences from three specimens of A. analyticus and A. octavius, two specimens of A. rupununi, and one specimen of A. ethicus and species of the genera Audifia sp. and Tekellina sp. I then combined these new sequences with previously published sequences from Agnarsson et al. (2007a, 2010). Genbank accession numbers of new sequences are not yet available. The total dataset contains 86 terminals, comprising 18 outgroups from across Theridiidae and 68 individuals representing 25 out of the 54 currently recognized Anelosimus species. Most of the missing Anelosimus species are outside the 'eximius lineage' (Agnarsson 2006), which contains most of the American species, including A. studiosus and relatives, and thus are not critical to the placement of the Uruguayan population. The data matrix is available from the author and will be submitted to the Dryad database (online at http://datadryad.org/).

I aligned and analyzed the molecular data using the same methods and settings as in previous studies (Agnarsson et al. 2007a, 2010). In summary, I aligned sequences in Clustal W (Thompson et al. 1994) with gap opening and extension costs of 24/6, followed by minor manual adjustments. I then concatenated the genes into a single five-gene matrix in Mesquite (Maddison & Maddison 2010) and exported them for model selection and analyses. The matrix was partitioned by gene, and for protein coding genes (COI, ND1), additionally by codon position, for a total of 9 partitions. The appropriate model for each partition was chosen in jModeltest 0.1.1 (Posada 2008), selecting only among the 24 models implemented in MrBayes. Final model choice for each partition was thus as follows: 28S, COI^{1st}, COI^{2nd}, 16S, $ND1^{2nd} = GTR+I+\Gamma$; COI^{3rd} , $ITS2 = GTR+\Gamma$; $ND1^{1st} =$ $HYK+\Gamma$; $ND1^{3rd} = HYK+I+\Gamma$. I then analyzed the concatenated matrix in MrBayes (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The Bayesian analysis was run for 10,000,000 generations, with all base frequencies estimated from the data and parameter estimates unlinked ("unlink statefreq = (all) revmat = (all) shape = (all) pinvar = (all)"). The first 5,000,000 were then discarded as 'burnin', after which stationarity had been reached.

Taxonomy.—Morphological methods were described in detail in Agnarsson (2004, 2006). Nomenclature of the male palpal organ follows Agnarsson et al. (2007b). All measurements are in millimeters and made using an Infinity K2 long-distance microscope. Prosoma and abdomen length and height are measured in lateral view, and widths in dorsal view are all measured at widest points. Leg segments are measured without the detachment of legs from the prosoma. Illustrations are prepared using a Visionary Digital imaging system,

the core components being a Canon 5D digital camera body and a K2 Infinity microscope equipped with Olympus metallurgical objectives. Successive images are combined with Helicon Focus 4.0, and thereafter minimally processed with Photoshop CS3 to adjust for both contrast and brightness and to remove background blemishes. For photography, anatomical preparations are temporarily mounted in alcohol-based hand sanitizer jelly (62% ethanol) and the specimen then covered with 70% ethanol. I deposited type specimens at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.; additional voucher specimens were lodged in the Zoological Museum, University of Puerto Rico, Río Piedras.

RESULTS AND DISCUSSION

Phylogenetics.—The new phylogeny in most details mirrors that of Agnarsson et al. (2007a, 2010), except in the placement of some species of the sclerotized CD clade, and in higher posterior probability support inferred for many nodes (Fig. 1). A studiosus (including specimens of A. tungurahua) plus the three Uruguayan specimens form a grade sister to other species of the sclerotized CD clade. Hence, the Uruguayan specimens are here described as a new species, A. vierae n. sp., and A. tungurahua is synonymized with A. studiosus (see below).

The newly added Chinese species A. chonganicus nests sister to species from Malaysia and Singapore within a clade, which, based on morphological evidence, also contains many African and Southeast Asian species; this clade is thus referred to as the 'Old World clade' (Fig. 1). Other newly added specimens of four additional Anelosimus species group as expected with their previously sequenced conspecifics, and all species here represented by multiple specimens are monophyletic (Fig. 1). The newly added genus Audifia (Hadrotarsinae) is used here as the primary outgroup, and its placement is thus not tested. However, the other newly added genus Tekellina does not group within the Theridiinae, unlike previously hypothesized (Agnarsson 2004). Further investigation of the placement of these two genera is necessary and will be facilitated by the sequences made available here.

Spiders of two genera, Anelosimus and Stegodyphus, are the major models in the study of spider sociality and its evolution (e.g., Avilés 1997; Avilés et al. 2000, 2001; Jones & Parker 2000, 2002; Bukowski & Avilés 2002; Johannesen et al. 2002, 2007, 2009; Powers & Avilés 2003, 2007; Bilde et al. 2005, 2007; Jones et al. 2007; Lubin & Bilde 2007; Purcell & Avilés 2007; Yip et al. 2008; Pruitt et al. 2008, 2010; Pruitt & Riechert 2009; Duncan et al. 2010). Discovery and phylogenetic placement of new species in these genera will deepen our understanding of social evolution and its causes and consequences. Because many aspects of the behavior of A. vierae are already studied, describing and phylogenetically placing this new species will contribute to the phylogenetic ancestral character reconstruction of the various components of social behavior in spiders. Furthermore, A. vierae is a close relative of the socially polymorphic A. studiosus and will thus represent a good model to complement recent studies on social polymorphism and its origin and consequences (Jones and Parker 2000, 2002; Jones et al. 2007; Pruitt et al. 2008, 2010; Pruit & Riechert 2009; Duncan et al. 2010).

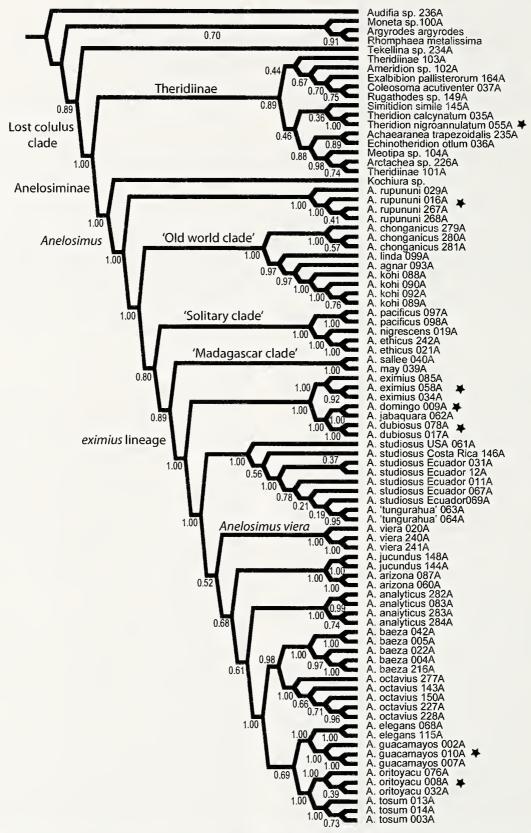


Figure 1.—Results of Bayesian phylogenetic analysis of the concatenated dataset for the genus *Anelosimus*. The new species *A. vierae* does not group with *A. studiosus*, where the Uruguayan specimens were tentatively placed before. Permanently social species are marked with \star .

All recent studies corroborate the 'maternal care route to sociality' (Avilés 1997; Agnarsson 2002, 2004; Bilde et al. 2005; Johannesen et al. 2007, 2009; Agnarsson et al. 2010), in which maternal care precedes subsociality, which in turn precedes quasisociality in evolutionary time. The study of intermediate social (subsocial) species is thus fundamental to understanding the evolution of quasisociality (e.g. see Powers & Avilés 2003).

Natural history.—This section summarizes previous work on the natural history and behavior of this species (Viera et al. 2006, 2007a, b, c; Albo et al. 2007; Viera & Albo 2008). Anelosimus vierae is a subsocial species, the mother of which cares for her young, and the juveniles show a lack of conspecific aggression, but rather cooperate in the natal nest until dispersal, near or at adulthood (Albo et al. 2007). Absence of aggression is nearly complete for motile instars; females cannibalize some eggs, but never eat larvae or nymphs (Viera et al. 2007c). Maternal care involves many elements, starting with egg-sac guarding and then opening the egg sac to release the young. The larvae are not able to break out of the egg sac by themselves (Viera et al. 2007c). Mothers open the egg sac based on time since laying the egg (21 days), but the mothers' actions are also triggered by the movement of nymphs within the egg sac (Viera et al. 2007c). The mother then feeds her offspring via regurgitation (Viera et al. 2005). The mother dies as the juveniles reach instars IV-VI. A very interesting feature of this species is that the juveniles then continue to feed each other via regurgitation (Viera et al. 2005). As a result of these altruistic acts, there is an equalizing of food distribution among colony members, which may prevent starvation and result in more individuals reaching adulthood. Although this remains to be observed in other species, it seems likely that juvenile food sharing may represent an evolutionary 'stepping-stone' towards permanent sociality.

In general, males of *A. vierae* mature earlier and consistently disperse, while females mature asynchronously and may or may not disperse from the natal nest. The consequence of this dispersal pattern is the formation of some multi-female nests. The occurrence of multi-female nests, in turn, implies a somewhat intermediate social structure, or social polymorphism, as seen in certain populations of *A. studiosus* in North America (Jones & Riechert 2008; Pruitt et al. 2008). In fact, the primary sex ratio in this species is also slightly female biased (2:1) (Viera et al. 2007a), implying some interdemic selection (Avilés 1993, 1997). Hence, *A. vierae* could be characterized as an intermediate social species, showing levels of sociality somewhere close to *A. jabaquara* (Marques et al. 1998).

The early-maturing males court and guard both subadult and adult females and fight other males, indicating competition for paternity among males (Albo et al. 2007). Fights can be repeated and males winning first fights may eventually lose to other males. Males court females using vibration, silk thread plucking, and touching the female until she adopts a copulation position. Males that lose fights may still remain as satellites around the nest and opportunistically mate with her later. This implies that strict first male priority need not be the rule in *A. vierae*, and this implication was recently confirmed through a gamma radiation sterilization experiment, showing that first and second males have about equal levels of paternity success (Lorieto et al. 2010).

TAXONOMY

Remarks.—Agnarsson (2004) established a classification of theridiid spiders, placing most of the genera into subfamily-level clades. However, *Anelosimus* and *Kochiura* remained unplaced. Here I find a well-supported clade including *Anelosimus* and *Kochiura*, which together form a clade sister to the subfamily Theridiinae. Therefore, the subfamily Anelosiminae is established to accommodate *Anelosimus*, *Kochiura*, and possible relatives of these taxa (Fig. 1). While naming well-supported clades is certainly useful, establishing many fixed ranks in between genera and families can be problematic (Kuntner & Agnarsson 2006), and authors should be careful not to treat theridiid subfamilies as 'comparable' taxonomic units; fair comparisons are between sister clades.

Family Theridiidae Sundevall 1833 **Subfamily Anelosiminae** subfam. nov.

Remarks.—Anelosiminae currently includes *Kochiura* and *Anelosimus*. A putative synapomorphy of this clade is the characteristic abdominal pattern (Figs. 6–8). These genera also have a unique combination of characters with colulus highly reduced (*Kochiura*) or absent (*Anelosimus*), but with two small colular setae usually present. This character combination, however, does not represent a synapomorphy, as the two taxa have different states of colular reduction, and the retention of two colular setae is primitive, which may explain why there is little support for this arrangement in morphological data. Furthermore, the character-rich palpal organ is extremely variable within this subfamily such that Anelosiminae synapomorphies are not evident. Anelosiminae is readily diagnosed from the sister subfamily Theridiinae, as in the latter all species lack colular setae.

Genus Anelosimus Simon 1891

Type species.—Anelosimus socialis Simon 1891 [= Anelosimus eximius (Keyserling 1884)].

Remarks.—See Agnarsson (2004, 2006) for taxonomic treatment of the genus.

Anelosimus studiosus (Hentz 1850)

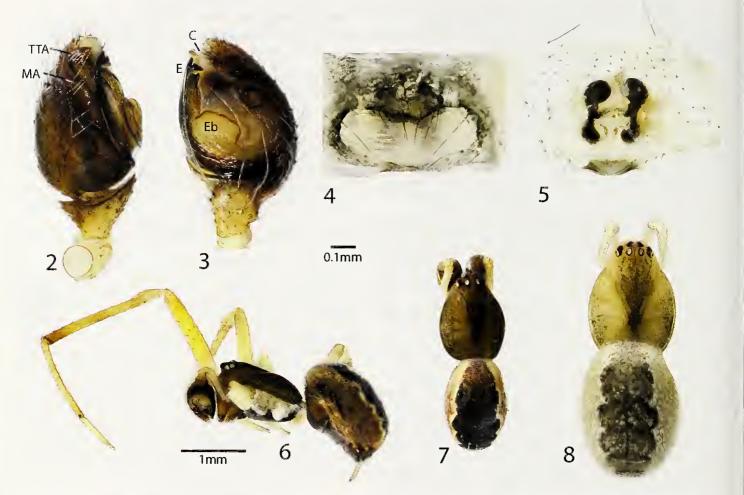
Anelosinius tunguraliua Agnarsson 2006:502, figs. 35K–Q, 42–43, 64D. New Synonymy.

Synonymies.—See Agnarsson (2006) for detailed synonymies Remarks.—Agnarsson's (2006) diagnosis was based on subtle morphological differences between *A. tungurahua* and *A. studiosus*, namely that males of the former had a flatter embolic division b (Eb), with a narrower and less rugose distal tip, and females had a larger epigynal lip. However, in light of the current results, these traits are now considered as intraspecific morphological variation of *A. studiosus*.

Auelosiums vierae new species (Figs. 2–8)

Material examined.—*Type:* Holotype &, URUGUAY: *Montevideo*, Montevideo, Melilla, 34.90°S, 56.15°W, 30 m, November 2003, C. Viera and F. Costa (USNM).

Other specimens (not types).—3 ♂, 3 ♀, same data (USNM); 1 ♂, 1 ♀, URUGUAY, *Lavalleja*, Sierra de Minas, Parque de Vacaciones, 34.426°S, 55.195°W, December 2005, W. Maddison, G. Ruiz, M. Simó, M.E. Rodriguez (USNM).



Figures 2–8.—Anelosimus vierae sp. n. genitalia and habitus of specimens from Sierra de Minas, Lavalleja, Uruguay. 2, 3: Male palp, ectal, ventral; 4, 5: Female epigynum, ventral, dorsal cleared; 6, 7: Male habitus, ectal, dorsal; 8: Female habitus, dorsal. Upper scale bar for Figs. 2–5, lower scale bar for Figs. 6–8.

Etymology.—The species epithet is a noun in apposition; a patronym after Carmen Viera, whose work on this species has revealed some fascinating behaviors, and inspired further investigation into its phylogenetic placement.

Diagnosis.—Males can be diagnosed from other *Anelosimus* species except *A. studiosus* by the sharp constriction of the embolic division b (Eb) centrally (Fig. 3), and from *A. studiosus* by longer distal arm of the Eb, and wider lightly sclerotized area separating the Eb from the ectal tegular margin. Females differ from all other *Anelosimus* species, except others in the *studiosus* group, by having the strongly sclerotized portion of the copulatory duct (see Agnarsson 2006) directly ventral to the spermathecae (Fig. 5). However, females are difficult to diagnose from other species of the *studiosus* group, except using molecular data.

Description.—*Male* (Sierra de Minas, Uruguay): Total length 2.80. Cephalothorax 1.35 long, 1.05 wide, 0.80 high, brown. Abdomen 1.45 long, 1.00 wide, 1.15 high. Pattern as in Figs. 6, 7. Eyes subequal in size about 0.08 in diameter. Chelicerae with one large and two small prolateral teeth, three to four denticles retrolaterally. Leg I femur 1.70, patella 0.45, tibia 1.65, metatarsus 1.35, tarsus 0.70. Femur not noticeably thickened, ventral thickened hairs on metatarsus one absent.

Leg formula 1243. Leg base color yellowish, light brown, with distal tip of femora and tibia darker. Four to five small trichobothria dorsally on all tibia. Trichobothria on metatarsi I–III proximal (about 0.35–0.40), absent on metatarsus IV. Palp (Figs. 2, 3) as in other species of the *studiosus* group, smaller and with less voluminous sclerites than species of the *jucundus* group. Embolus spiral runs along mesal margin of palp terminating in a ridged bifurcation, embolus with a simple flat, embolic division b, which is narrow distally. The basal lobe of the embolus is oriented toward the subconductor, from which a small and translucent conductor arises. Median apophysis simple, without ducts, interacting with cymbial hood. TTA hooked and ridged distally.

Female (Sierra de Minas, Uruguay): Total length 3.70. Cephalothorax 1.80, long, 1.40 wide, 1.00 high, brown. Abdomen 2.10 long, 1.55 wide, 1.30 high. Pattern as in Fig. 8. Eyes subequal in size, about 0.10 in diameter. Chelicerae with one large and two small prolateral teeth, three denticles retrolaterally. Leg I femur 2.10, patella 0.65, tibia 1.80, metatarsus 1.70, tarsus 0.90. Leg formula 1243. Leg base color light yellowish-brown, with distal tip of tibia darkened. Four to seven small trichobothria dorsally on all tibia. Trichobothria on metatarsi I–III central or slightly

proximal (about 0.45–0.50), absent on metatarsus IV. Four to five dorsal trichobothria on female palpal tibia. Epigynum externally a lightly ridged plate, internally with simple short copulatory and fertilization ducts, copulatory ducts strongly sclerotized and situated directly below the ectalmost margin of the spermathecae (Figs. 4, 5).

Variation.—Female total length 3.60–4.20, male total length 2.5–2.85.

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A new species of the genus *Idiops* and notes on *Idiops bombayensis* Siliwal et al. 2005 (Araneae: Idiopidae) from Northern Western Ghats of Maharashtra, India

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Abstract. A new species of trapdoor spider, *Idiops rubrolimbatus* sp. nov., is described from the northern part of the Western Ghats of Maharashtra. *Idiops bombayensis* Siliwal et al. 2005 was originally described based on only a female specimen. Here, a description of the male is provided for the first time, along with a female description based on fresh collections from Mumbai and Matheran, Maharashtra state, India. Natural history information is provided for both species.

Keywords: Trapdoor spider, male, natural history, burrow

In India, trapdoor spiders are represented by four families, and the most widespread and species-rich family is the Idiopidae (Siliwal et al. 2005, Siliwal et al. 2009). Worldwide, this family is represented by 22 genera and 303 species in three subfamilies (Platnick 2011) and in India by three genera, namely Heligmomerus Simon 1892, Idiops Perty 1833 and Scalidognathus Karsch 1891, totaling 12 species (Siliwal et al. 2007; Sanap & Mirza 2011). The genus Idiops Perty 1833 is the most widespread trapdoor genus, being represented by seven species in India (Siliwal et al. 2005; Siliwal et al. 2010). This genus was originally placed in the Ctenizidae; but Raven (1985) transferred it to the Idiopidae, differentiating males of the Idiopidae from those of the ctenizids in having a distal haematodocha extending almost down to the embolus, transforming the distal sclerite into an open scoop, and also by the bilobed palpal tarsus with one blunt and one acutely pointed lobe. Many species of Acanthodon Guérin 1838 were transferred to Idiops (see Platnick 2011). Recently, Siliwal et al. (2010) transferred two species, I. biharicus and I. barkudensis, from Idiops to Heligmomerus.

Idiops bombayensis Siliwal et al. 2005 was described by Pocock (1899) from the 'Bombay region' as Acanthodon opifex and later was transferred to Idiops by Roewer (1942). This transfer created a homonymy with Idiops opifex Simon 1899; thus to stabilize the nomenclatural conflict, Siliwal et al. (2005) provided a replacement name for I. bombayensis. This species was known from the Bombay (= Mumbai) region without any precise locality, and the original description was based on few prominent morphological characters, lacking information like leg morphometry, spermathecae and natural history. While conducting surveys in and around Mumbai, we collected specimens of two species of the genus Idiops. We identified one of them as I. bombayensis, and the other one represents a hitherto undescribed species. In the present paper, we provide detailed taxonomic descriptions of both sexes of I. bombayensis based on the fresh collection along with the description of the new species.

METHODS

Spiders were collected during biodiversity surveys conducted in 2010 in Mumbai and Matheran, Maharashtra. The

specimens are deposited at the Wildlife Information Liaison Development Society, Coimbatore, Tamil Nadu. Measurements of body parts except for the eyes were taken with a MitutoyoTM Dial Caliper. Eye measurements were done with a calibrated ocular micrometer. All measurements are in mm. Spermathecae were dissected and cleared in clove oil using teasing needles. Specimens were examined using a LabomedTM CSM2 stereo-binocular microscope. Descriptive style follows Siliwal et al. (2009). All illustrations were prepared using a camera lucida attached to a CETIITM stereomicroscope by MS. The description was compared with available literature by Pocock (1900) and Tikader (1977).

Abbreviations: ALE = anterior lateral eye, AMC = Aarey Milk Colony, AME = anterior median eye, MOQ = median ocular quadrate, PLE = posterior lateral eye, PME = posterior median eye, PLS = posterior later spinnerets, PMS = posterior median spinnerets, WILD = Wildlife Information Liaison Development Society, RS = Rajesh Sanap, ZM = Zeeshan Mirza. Abbreviations used for hair and spine counts are d = dorsal, fe = femur, mt = metatarsus, p = prolateral, pa = patella, r = retrolateral, ta = tarsus, ti = tibia and v = ventral.

TAXONOMY

Idiops Perty 1833

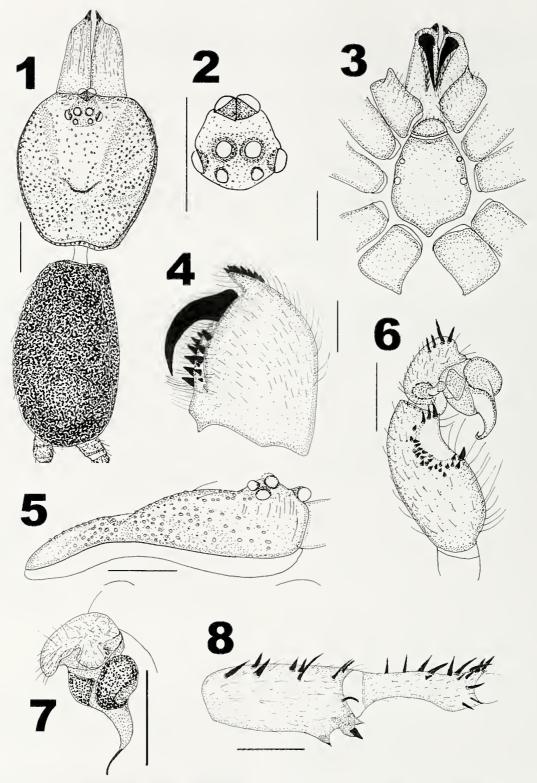
Idiops Perty 1833:197; Gravely 1915:261; Gravely 1935:69;
Raven 1985:138; Dippenaar-Schoeman 2002: 68.
Acanthodon Guérin 1838:10; Simon 1892:91; Pocock 1900:161;
Tikader 1977:306.

Type species.—Idiops fuscus Perty 1833.

Diagnosis.—ALE set far in advance of others, making eye group much longer than wide; chelicerae medially normal; dorsal abdomen soft, lacking chitinized shield; two rows of cheliceral teeth and posterior sternal sigilla absent (Raven 1985).

Idiops bombayensis Siliwal, Molur & Biswas 2005 (Figs. 1-15)

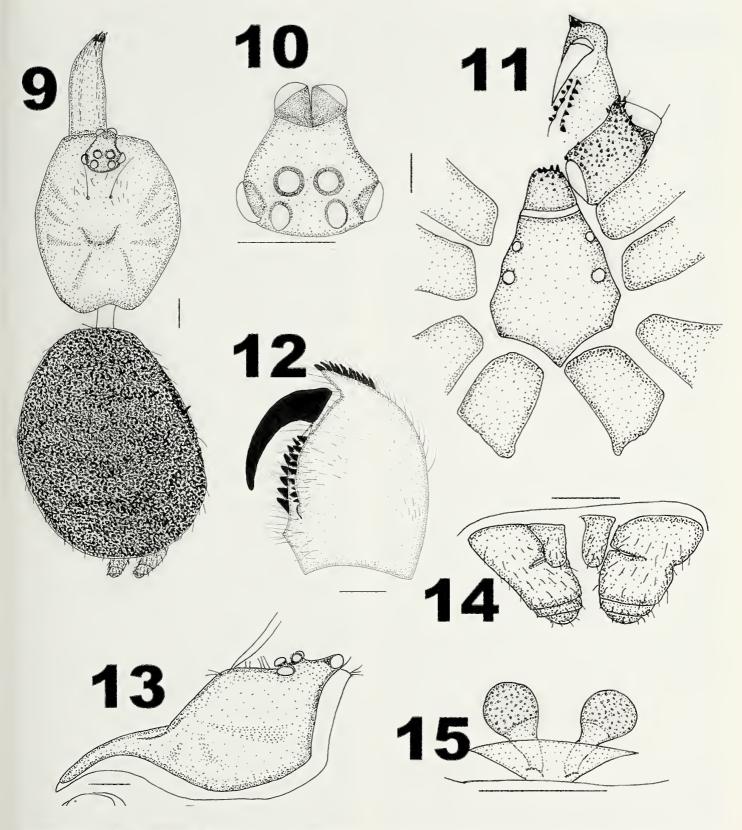
Acanthodon opifex Pocock 1899:750, 1900:162. Idiops bombayensis Siliwal, Molur & Biswas 2005:2004.



Figures 1–8.—*Idiops bombayensis* male from Aarey Milk Colony. 1. Cephalothorax and abdomen, dorsal view; 2. Eyes; 3. Sternum, labium, maxillae and chelicerae; 4. Chelicerae prolateral view; 5. Lateral view of carapace; 6. Palp tarsi and tibia, retrolateral view; 7. Palp bulb, ventral view; 8. Metatarsi and tibia of leg I. Scale bar 1mm.

Type material.—Holotype, female, Bombay, coll. H.M. Phipson, Museum of Natural History, London (not examined). Material examined.—INDIA: *Maharashtra*: Raighad district, Matheran, 19°00′N, 73°17′E, 19 February 2010, coll. Rajesh Sanap, Ashish Jadhav & Zeeshan Mirza, 1 female,

WILD-10-ARA-401; Mumbai, Aarey Milk Colony. 19°07′31″N, 72°52′76″E, 26 February 2010, coll. Rajesh Sanap & Zeeshan Mirza, 1 female, WILD-10-ARA-402; 1 male, 27 June 2010, coll. Rajesh Sanap & Zeeshan Mirza, WILD-10-ARA-545.



Figures 9–15.—*Idiops bombayensis* female from Aarey Milk Colony. 9. Cephalothorax and abdomen, dorsal view; 10. Eyes; 11. Sternum, labium, maxillae and chelicerae; 12. Chelicerae prolateral view; 13. Carapace lateral view; 14. Spinnerets; 15. Spermathecae. Scale bar 1 mm.

Table 1.—Morphometry of legs and palp of the female (WILD-07-ARA-401), (WILD-07-ARA- 402) and male (WILD-07-ARA- 545) of *Idiops bombayensis*. All measurements in mm (\pm 0.02 mm).

	Leg I			Leg II			Leg III			Leg IV			Palp		
	#401	#402	#545	#401	#402	#545	#401	#402	#545	#401	#402	#545	#401	#402	#545
Femur	4.42	3.26	2.94	3.68	2.74	2.58	3.56	2.68	1.96	5.22	3.86	2.86	3.98	2.60	1.72
Patella	2.42	1.68	1.12	2.36	1.90	0.98	2.56	1.56	0.84	3.30	2.02	1.28	2.18	1.48	0.68
Tibia	2.52	1.94	1.92	2.30	1.36	1.74	1.82	1.54	1.14	3.32	2.15	2.04	2.44	2.04	1.58
Metatarsus	2.18	1.28	1.94	2.18	1.52	1.50	2.18	1.20	1.28	2.94	1.48	1.92	_	_	_
Tarsus	1.08	0.94	0.98	1.42	1.28	0.76	1.52	0.84	0.76	1.68	1.15	1.06	2.70	1.66	0.50
Total	12.64	9.1	8.9	11.94	8.8	7.56	11.64	7.82	5.98	16.64	10.66	9.16	11.3	7.78	4.48
Midwidth															
Femur	1.42	1.14	0.60	1.38	1.40	0.62	1.68	1.16	0.78	1.96	1.30	0.72	1.42	1.02	0.32
Tibia	1.06	0.84	0.84	1.04	0.82	0.44	1.30	0.88	0.44	1.40	0.78	0.44	1.04	0.82	0.76

Diagnosis.—*Idiops bombayensis* males differ from those of *I. fossor*, *I. designates*, *I. rubrolimbatus* and *I. garoensis* in possessing a prominent tubercle below the tibial apophysis. Females differ from those of *I. fortis* and *I. constructor* in lacking a band of spinules below coxa IV and in having the tibia of leg III longer than wide, and from those of *I. madrasensis* in spermathecae shape.

Description.—Male from Aarey Milk Colony (WILD-10-ARA-545). Total length 8.06; carapaee 3.54 long, 2.74 wide; chelicerae 3.18 long. Abdomen 4.52 long, 3.12 wide. Spinnerets: PMS, 0.18 long, 0.10 wide, 0.30 apart; PLS, 0.76 total length (0.38 basal, 0.24 middle, 0.14 distal; midwidths 0.38, 0.30, 0.18 respectively). Morphometry of legs and palp are given in Table 1.

Color in life (Fig. 36): overall blackish; carapace deep glossy black, abdomen reddish brown. Anterior legs blackish brown, except for the tarsi and distal portion of the metatarsi; posterior legs paler and more brownish.

Carapace (Figs. 1, 5): reddish-brown, granules/tubercles, dense, throughout earapace; two long and several short bristles on caput; few lines of depression along interstitial ridges. Caput with distinct mound between fovea and eyes, rough. Fovea deep, procurved, U-shaped.

Eyes (Fig. 2): eight, ALE situated far in advance of rest. Posterior row slightly procurved, ocular group 1.60 long, 0.56 wide; diameter AME 0.14, PME 0.10, ALE 0.14, PLE 0.12; distance between ALE-AME 0.22, AME-AME 0.08, PLE-PME 0.06, PME-PME 0.20; MOQ not square, 0.38 long, 0.34 front width, 0.36 back width.

Maxillae (Fig. 3): 0.98 long in front and 1.10 long in back, 0.68 wide; cuspules absent, anterior lobe distinct.

Labium (Fig. 3): 0.42 long, 1.48 wide; labiosternal groove shallow, cuspules absent.

Chelicerae (Fig. 4): 5 promarginal teeth and 6 retromarginal teeth; rastellum conspicuous on distinct process; 10 large and small spines on dorso-prolateral; ventral face and up.

Sternum (Fig. 3): yellowish-green, with elevated anterior and lateral sides, sloping posteriorly, 1.60 long, 1.54 wide, covered with long black hair, row of these radiating out of borders, posterior angle acute.

Sigilla (Fig. 3): anterior 0.8 in diameter and 0.84 apart, situated 0.02 from margin; middle ca. 0.10 in diameter and 1.20 apart and 0.06 away from margin; posterior sigilla absent.

Legs: leg I clearly thicker than rest, greenish-brown above and light yellowish-green below. Metatarsi of all legs longer than tarsi. Two conspicuous hairless bands running for length of femora, patellae and tibiae. Tibia I, with apophysis with a triangular stout spine below, with a tubercle with a pointed spine; mt I deeply incrassate in basal 3/4, with indistinct prolateral process (Fig. 8). Scopulae, tibia I–III present, full length, ti IV absent; claw tufts absent. Leg formula 1423.

Spines: curved, thick thorn-like or stout spike-like spines. ti I, p=2, r=2; mt I, r=8; ta I, r=5; ti II, p=2, r=3; mt II, p=2, r=5; ta II, p=1, r=1; pa III, r=2; ti III, r=2, v=2; mt III, r=5, v=2; fe III, r=2; pa IV, p=9; ti IV, v=4; mt IV, p=2, v=4; ta IV, p=3, v=2; palp, ti, v=25; ta, v=25

Coxae: yellowish-green; coxa IV wider than rest; coxa I longer than rest.

Claws: all legs with three claws, paired claws I-III with 5 small teeth and IV with 3 teeth.

Abdomen (Fig. 1): glossy reddish-brown with silvery golden spike-like hairs in life; in alcohol, grayish-brown with yellowish dots dorsally; covered with short and long black hairs; ventrally yellowish-green covered with black hairs.

Spinnerets: PMS digitiform; PLS, apical segment domeshape. Overall covered with brown hair and with numerous spigots on all segments.

Palp (Fig. 6, 7): tibia inflated with ventral concavity, crescent band of 26 spines on retrolateral side of concavity. Tarsus bilobed, one lobe blunt and another digitiform, dorso-distally four spines. Palp simple, embolus broad at base tapering abruptly at distal end; distal end twisted and embolus tip facing towards the retrolateral aspect and forward; terminates in scoop-like structure.

Description.—Female from Aarey Milk Colony (WILD-10-ARA-402). Total length 15.88; carapace 5.88 long, 7.02 wide; chelicerae 3.68 long. Abdomen 10.0 long, 7.02 wide. Spinnerets: PMS, 0.72 long, 0.20 wide, 0.08 apart; PLS, 2.06 total length (0.68 basal, 0.92 middle, 0.46 distal; midwidths 0.94, 0.80, 0.68 respectively). Morphometry of legs and palp are given in Table 1.

Color in life (Fig. 37): glossy blackish brown all over. Chelicerae and dorsal aspect of legs black. Abdomen dark brown.

Carapace (Figs. 9, 13): yellowish-brown, glabrous except for two long and short spine-like hairs on caput, few lines of

depression along interstrial ridges. Caput with distinct mound between fovea and eyes. Fovea deep, proeurved, U-shaped.

Eyes (Fig. 10): eight, ALE situated far in advance of rest. Posterior row slightly procurved, ocular group 0.88 long, 0.86 wide; diameter AME 0.20, PME 0.08, ALE 0.14, PLE 0.16; distance between ALE-AME 0.30, AME-AME 0.12, PLE-PME 0.08, PME-PME 0.22, ALE-ALE 0.08, ALE-PLE 0.58; MOQ not square, 0.40 long, 0.46 front width, 0.58 back width.

Maxillae (Fig. 11): 1.98 long in front and 1.23 long in back, 1.26 wide; ca. 75 cuspules on anterior edge larger than rest. Anterior lobe distinct.

Labium (Fig. 11): 0.96 long, 1.08 wide, labiosternal groove shallow anteriorly, cuspules arranged in two, 5 large in first row and 4 small in a row behind the large cuspules.

Chelicerae (Fig. 12): 6 promarginal and 6 retromarginal teeth, basomesal teeth absent; rastellum eonspicuous on distinct process, 20 spines dorso-prolateral, vertical face and up.

Sternum (Fig. 11): yellowish-brown, with elevated anterior and lateral sides, sloping posteriorly, 3.26 long, 2.62 wide, covered with long black hair, row of these radiating out of borders, posterior angle acute.

Sigilla (Fig. 11): anterior 0.18 in diameter and 2.10 apart, situated on margin; middle about 0.22 in diameter and 2.40 apart; posterior sigilla absent.

Legs: leg III clearly thicker than rest, brownish-green above and yellowish-green below, except tarsi of palp and metatarsi and tarsi of all legs darker above. Metatarsi of all legs longer than tarsi. Two conspicuous hairless bands running for length of femora, patellae and tibiae. Scopulae and claw tufts absent on tarsi of all legs and palp. Leg formula 4123.

Spines: curved, thick thorn-like and normal spines. ti I, p = 14, r = 14; mt I, p = 18, r = 20; ta I, p = 10, r = 9; ti II, p = 8, r = 4; mt II, p = 19, r = 7; ta II, p = 7, r = 4; pa III, p = 1, r = 2; ti III, p = 9, r = 3; mt III, p = 7, r = 5; ta III, p = 6, v = 6; pa IV, p = 32; mt IV, p = 8, v = 1; ta IV, p = 10, v = 2; palp, fe, p = 1, pa, p = 1; ti, p = 18, r = 15; ta, p = 21, r = 24.

Coxae: coxae of legs yellowish-brown; coxae IV wider than rest; coxae I longer than rest.

Claws: all legs with three claws, paired claw I with single tooth; II–IV with two teeth. Palp with single claw bearing single unequal tooth. Claws of leg IV longer than rest, claws of leg I & II equal, claw of leg III smallest. Claw tufts absent.

Abdomen (Fig. 9): glossy blackish-brown with silvery-golden spike-like hairs in life, grayish-brown dorsally; covered with short and long setae; ventrally yellowish-grey eovered with brown hairs.

Spinnerets (Fig. 14): PMS digitiform; PLS, covered with brown hair, apical segment dome-shape. Covered with brown hair and numerous spigots.

Spermathecae (Fig. 15): globular apical lobe on stalk, resembling button mushroom.

Variation.—morphometry of specimen from Matheran (WILD-10-ARA-401). Total length 16.84; carapaee 6.74 long, 5.72 wide; chelicerae 4.02 long, 8 retromarginal and 9 promarginal teeth. Sternum 3.98 long, 3.44 wide. Labium 1.12 long, 1.68 wide, 4 large cuspules in 2 rows (2 + 2). Maxillae 1.12 long back, 1.98 long front, 1.26 wide, cuspules 60–80 of varying size, larger near the promarginal region. Abdomen 10.10 long and 7.34 wide. Spinnerets: PMS, 0.60

long, 0.40 wide, 0.28 apart; PLS, 1.74 total length (0.66 basal, 0.96 middle, 0.12 distal; midwidths 0.88, 0.78, 0.68 respectively). Morphometry of leg and palp given in Table 1.

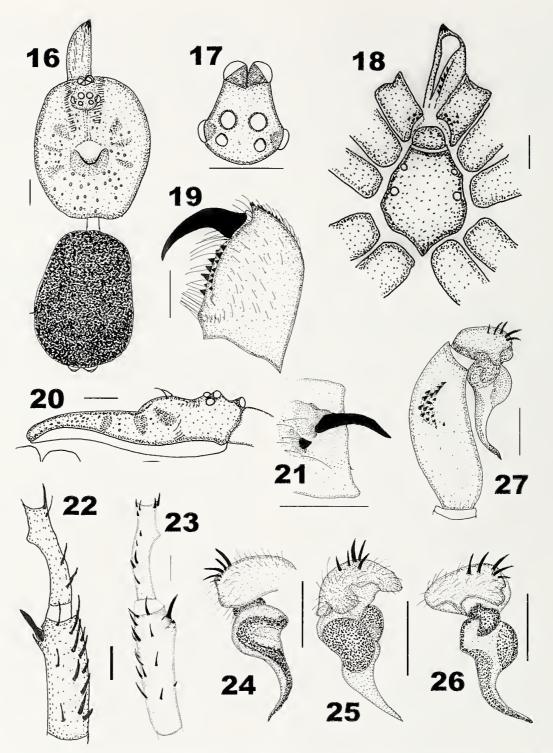
Eyes: eight, ALE situated far in advance of rest. Posterior row slightly procurved, ocular group 1.34 long, 1.28 wide; diameter AME 0.22, PME 0.10, ALE 0.24, PLE 0.18; distance between ALE-AME 0.74, AME-AME 0.18, PLE-PME 0.12, PME-PME 0.50, ALE-ALE 0.16, ALE-PLE 0.86; MOQ not square, 0.54 long, 0.56 front width, 0.62 back width.

Natural history and distribution.—Spiders were collected from Aarey Milk Colony, Mumbai and Matheran, Raighad district in Maharashtra state (Fig. 35). Burrows of this species have also been observed in the Sanjay Gandhi National Park in Mumbai. Habitat in Mumbai is of typical mixed moist deciduous forest and that in Matheran of semi-evergreen type. The flora in Mumbai region is composed of *Tectona grandis*, Butea monosperma, Cassia sp., Bombax sp., Acacia spp., Ziziphus spp. and several exotic species. Most of the burrows (especially of juveniles) were found on roadside mud bunds and a few under shrubs or at the base of trees (large individuals). The density of these spiders was about 8-20 per m². The burrow structure was a simple trapdoor, a single or sometimes double entrance leading to a tubular burrow, which was wider at base than at its entrance. The burrow and the inner side of the door were lined with a thick layer of white silk. The 'D' shaped doors were made up of thick layer (5 mm) of mud, moss or lichen, which were supported by a thick layer of silk, making them well camouflaged with their surrounding. The diameter of the burrow of the specimen from Matheran at the door was about 13 mm and the chamber 15 mm inside. All the burrows were observed to be perpendicular to the angle of the slope of the roadside bunds. The burrows ranged from 60– 200 mm in total length. Several burrows were found empty with an empty egg sac (probably juveniles had hatched out and dispersed) in the first week of May, and two burrows had females with intact egg sacs. The eggs sacs were dissected to estimate brood size. The first egg sac contained 32 juveniles collected from the burrow of a small female and the other with 155 juveniles excavated from the burrow of a large female. This species is presently known with certainty from only three localities: Matheran (Raighad district), Mumbai and Bhimashankar (Pune district). After heavy rains in June in Mumbai, several female specimens were found in leaf litter. Soil erosion and removal of soil for brick making is the major threat to this species at the collection localities.

Idiops rubrolimbatus new species (Figs. 16–34)

Type specimens.—INDIA: *Maharashtra*: holotype male, Aarey Milk Colony near Royal Palms, Mumbai, 19°07′31.94″N, 72°52′76.87″E, 12 May 2010, Rajesh Sanap and Zeeshan Mirza (WILD-10-ARA-1108); one female paratype, same data as holotype (WILD-10-ARA-1109).

Diagnosis.—*Idiops rubrolimbatus* male differs from those of *I. constructor* and *I. bombayensis* in lacking a large tubercle below the tibial spur, differs from *I. designatus* in having a slender and distinctly concave metatarsus of leg I, from *I. garoensis* and *I. bombayensis* in possessing cuspules on the maxillae and labium. Differs from *I. fossor* in having moderate



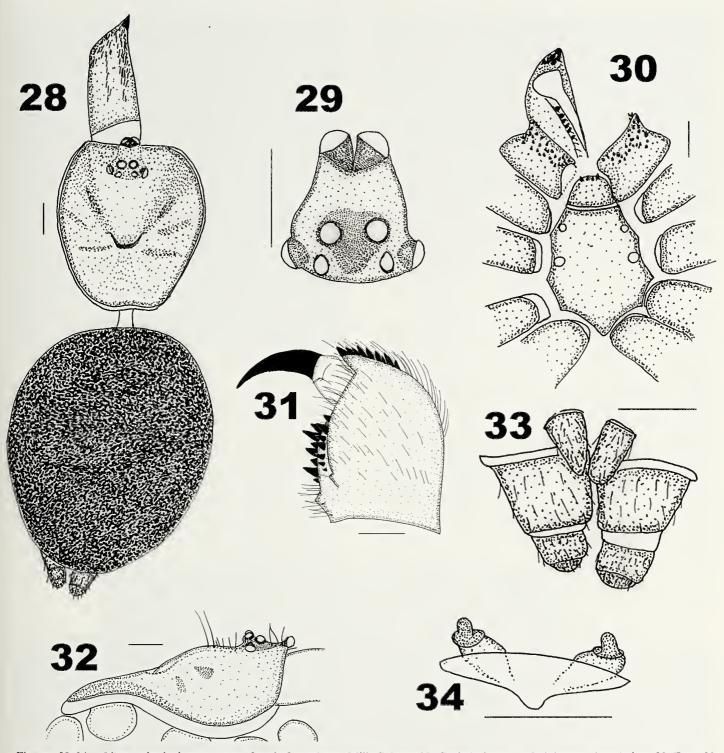
Figures 16–27.—*Idiops rubrolimbatus* sp. nov. male from Aarey Milk Colony. 16. Cephalothorax and abdomen, dorsal view; 17. Eyes; 18. Sternum, labium, maxillae and chelicerae; 19. Chelicerae prolateral view; 20. Carapace lateral view; 21. Tibial spur; 22. metatarsus and tibia of leg I; 23. Metatarsus and tibia of leg I; 24. Palp bulb, prolateral view; 25. Palp bulb, frontal view; 26. Palp bulb, retrolateral view; 27. Palp tarsi and tibia, retrolateral view. Scale bar 1 mm.

concavity on metatarsi that exceeds half the length of the segment (strong concavity in basal half of metatarsi in *I. fossor*), tip embolus faces outward and toward the retrolateral face (tip of embolus faces forward and toward the retrolateral face in *I. fossor*).

Females of *I. rubrolimbatus* differ from those of *I. constructor* and *I. fortis* in lacking a band of spinules under

coxae IV. Spermathecae emerging from distal ends of each leaf-like sclerotized structure fused at base supporting an inverted bell on a stalk distinguishing it from *I. bombayensis* and *I. madarasensis*.

Description.—Holotype male from Aarey Milk Colony (WILD-10-ARA-1108). Total length 10.88; carapace 5.38 long, 4.84 wide; chelicerae 2.84 long. Abdomen 5.50 long,



Figures 28–34.—*Idiops rubrolimbatus* sp. nov. female from Aarey Milk Colony. 28. Cephalothorax and abdomen, dorsal view; 29. Eyes; 30. Sternum, labium, maxillae and chelicerae; 31. Chelicerae prolateral view; 32. Carapace lateral view; 33. Spinnerets; 34. Spermathecae. Scale bar 1 mm.

4.06 wide. Spinnerets: PMS, 0.38 long, 0.20 wide, 0.24 apart; PLS, 0.79 total length (0.44 basal, 0.18 middle, 0.16 distal; midwidths 0.58, 0.44, 0.30, respectively). Morphometry of legs and palp given in Table 2.

Color in life (Fig. 38): carapace brownish with a red tinge on the periphery, abdomen blackish. Legs reddish brown overall.

Carapace (Figs. 16, 20): reddish-brown, warty along the interstitial ridges; two long and several short spine-like hairs on caput, few lines of depression along interstitial ridges. Caput with distinct mound between fovea and eyes. Fovea deep, procurved, U-shaped.

Eyes (Fig. 17): ALE situated far in advance of rest. Posterior row slightly procurved, ocular group 1.18 long,

Table 2.—Morphometry of legs and palp of holotype male	(WILD-07-ARA-1108) and female paratype (WILD-07-ARA-1109) of <i>I</i> .
rubrolimbatus sp. nov. All measurements in mm. (± 0.02 mm).	

	Leg I		Leg	I1	Leg III		Leg IV		Palp	
	#1108	#1109	#1108	#1109	#1108	#1109	#1108	#1109	#1108	#1109
Femur	5.99	3.66	5.04	3.5	3.57	3.42	5.2	4.55	3.78	3.58
Patella	2.91	2.46	2.37	2.33	2.33	2.53	2.93	3.2	2.11	2.49
Tibia	3.99	2.28	3.38	1.99	2.13	1.84	4.15	3.08	3.13	2.57
Metatarsus	4.56	1.99	3.99	1.64	3.5	2.08	4.71	3.14	0	0
Tarsus	1.65	1.01	2.15	1.06	2.08	1.41	2.22	1.67	3.01	2.73
Total	19.09	11.4	16.93	10.52	13.61	11.28	19.19	15.64	12.03	11.36
Midwidth										
Femur	1.5	0.98	1.36	1.1	1.42	1.62	1.46	1.3	0.8	0.76
Tibia	1.36	1.18	1.04	0.96	1.14	1.19	1.06	1.2	1.18	0.98

1.18 wide; diameter AME 0.20, PME 0.18, ALE 0.20, PLE 0.24; distance between ALE-AME 0.48, AME-AME 0.22, PLE-PME 0.08, PME-PME 0.34, ALE-ALE 0.22; MOQ not square, 0.46 long, 0.62 front width, 0.80 back width.

Maxillae (Fig. 18): 1.18 long in front and 1.70 long in back, 0.96 wide; 11 cuspules toward anterior inner edge, anterior lobe distinct.

Labium (Fig. 18): 0.58 long, 0.86 wide, labiosternal groove shallow, 3 large and 1 small cuspules on anterior edge.

Chelicerae (Fig. 19): 7 promarginal teeth and 6 retromarginal teeth; rastellum conspicuous on a distinct process, 15 spines on dorso-prolateral and vertical face and up.

Sternum (Fig. 18): yellowish brown, with elevated anterior and lateral sides, sloping posteriorly, 3.32 long, 2.62 wide, covered with long black hair, a row of these radiating out of the borders, posterior angle acute and not separating coxae IV.

Sigilla (Fig. 18): anterior 0.14 in diameter and 1.32 apart, situated 0.06 from margin; middle ca. 0.16 in diameter, 1.66 apart and 0.12 away from margin; posterior sigilla absent.

Legs: all legs reddish brown in life and orange in alcohol. Tibiae and femorae IV wider than rest. Metatarsi of all legs longer than tarsi. Coxae yellowish-brown. Two conspicuous hairless bands running for length of femora, patellae and tibiae. Leg formula 4123. Ti I, prolateral apophysis consists of a long spine with a small spine below it; mt I 3/4th incrassate, with a distinct short prolateral process (Figure 21–23). Scopulae present on ta I–III, absent on ta IV; claw tufts absent.

Spines: curved thick thorn-like or stout spike-like spines. pa I, v=4; ti I, p=5, r=3, v=10; mt I, p=1, r=6, v=1; ta I, p=5, r=5; pa II, p=1, d=1; ti II, p=4, r=4, v=7; mt II, p=4, r=8, v=2; ta II, p=1, r=7; pa III, p=8, r=3; ti III, p=8, r=11, v=5; mt III, p=7, r=10, v=3;

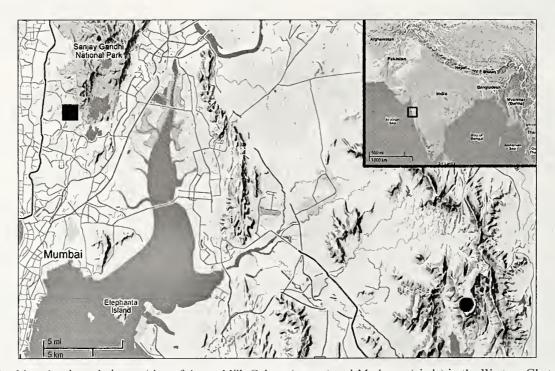


Figure 35.—Map showing relative position of Aarey Milk Colony (square) and Matheran (circle) in the Western Ghats of India.



Figure 36.—Idiops bombayensis male from Aarey Milk Colony.

fe III, d = 1; ta III, p = 1; fe IV, d = 4; pa IV, p = 15, d = 3; ti IV, p = 1, v = 5; mt IV, p = 5, v = 2, v = 7; ta IV, p = 4, v = 4, v = 6; palp, fe, d = 5; ti, r = 17; ta, d = 4.

Coxae (Fig. 18): IV wider than rest; I longer than rest.

Claws: all legs with three claws, paired claws of leg I & II with five teeth; claw of leg III with 2 and of leg IV with 4 teeth. Claw of leg IV longer than rest, claw of leg I & II equal, claw of leg III smallest. Claw tufts absent.

Abdomen (Fig. 16): reddish brown above; covered with short and long black hairs. Ventrally yellowish covered with black hairs. The preserved specimen wrinkled. Glossy reddish brown, with silvery golden spike-like setae in life.



Figure 37.—Idiops bombayensis female from Matheran.

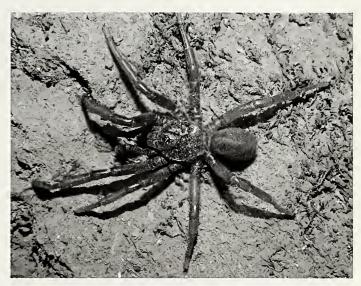


Figure 38.—Idiops rubroliubatus male holotype.

Spinnerets: PMS digitiform; PLS, apical segment domeshape (Fig. 5). Covered with brown hair and numerous spigots.

Palp (Figs. 24–27): tibia cylindrical (not inflated as seen in *I. bombayensis* and *I. constructor*) and lacks anterio-ventral concavity; retolaterally a small band of 16 spines on slightly elevated area, and one spine slightly away from the band. Tarsus, dorsally with three spines. Palp simple, embolus broad at base, gradually tapering and terminating in pointed scoop with slight twist; tip of embolus directed toward retrolateral face and projecting downwards.

Description.—Female paratype from Aarey Milk Colony (WILD-10-ARA-1109). Total length 14.16; carapace 6.10 long, 5.10 wide; chelicerae 3.12 long. Abdomen 8.06 long, 6.40 wide. Spinnerets: PMS, 0.48 long, 0.32 wide, 0.16 apart; PLS, 1.48 total length (0.24 basal, 0.74 middle, 0.50 distal; midwidths 0.80, 0.70, 0.54, respectively). Morphometry of legs and palp are given in Table 2.

Color in life (Fig. 39): Shade of brown overall, chelicerae deep black. Legs with reddish tinge.



Figure 39.—*Idiops rubrolimbatus* female from Sanjay Gandhi National Park (not collected).

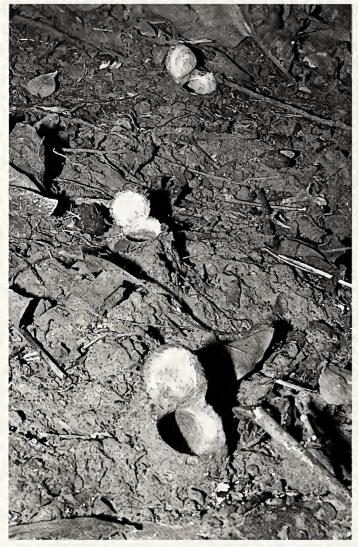


Figure 40.—Trapdoor burrows of *Idiops rubrolimbatus* from Sanjay Gandhi National Park.

Carapace (Figs. 28, 32): yellowish-brown, glabrous except for two long and short spine-like hairs on caput, few lines of depression along interstrial ridges. Caput with distinct mound between fovea and eyes. Fovea deep, procurved, U-shaped.

Eyes (Fig. 29): eight, ALE situated far in advance of rest. Posterior row slightly procurved, ocular group 1.30 long, 1.04 wide; diameter AME 0.18, PME 0.14, ALE 0.22, PLE 0.22; distance between ALE-AME 0.48, AME-AME 0.20, PLE-PME 0.10, PME-PME 0.38; MOQ not square, 0.38 long, 0.44 front width, 0.48 back width.

Maxillae (Fig. 30): 1.58 long in front and 2.30 long in back, 1.06 wide; with from ca. 50 cuspules. Cuspules towards anterior edge larger. Anterior lobe distinct.

Labium (Fig. 30): 0.98 long, 1.06 wide, labiosternal groove shallow; 4 cuspules anteriorly in single row.

Chelicerae (Fig. 31): 8 promarginal teeth and 7 retromarginal teeth, basomesal teeth absent; rastellum conspicuous on distinct process, 17 spines on dorso-porlateral, vertical face and up.

Sternum (Fig. 30): yellowish-brown, with elevated anterior and lateral sides, sloping posteriorly, 3.74 long, 2.88 wide,

covered with long black hair, row of these radiating out of borders, posterior angle acute.

Sigilla (Fig. 30): anterior, diameter 0.14 and 1.32 apart, marginal; middle, diameter ca. 0.22 and 1.86 apart, distance from margin 0.12; posterior sigilla absent.

Legs: Leg IV clearly thicker than rest, yellowish-brown above and light yellowish below, except tarsi of palp and metatarsi and tarsi of all legs darker above. Femora III clearly wider than rest. Metatarsi of all legs longer than tarsi. Two eonspicuous hairless bands running for length of femora, patellae and tibiae. Scopulae and claw tufts absent on tarsi of all legs and palp. Leg formula 4132.

Spines: curved thick thorn-like or stout spike-like spines. ti I, p=11, r=11; mt I, p=15, r=19; pa = 4, ta I, p=5, r=6, v=6; ti II, p=6, r=4; mt II, p=14, r=6, v=1; ta II, p=5, r=3, v=2; pa III, p=8, r=3; ti III, p=6, r=7; mt III, p=10, r=10; ta III, v=10; pa IV, p=14; fe IV, p=2; mt IV, p=4, v=3; ta IV, p=5, v=7; palp, fe, p=2, pa, p=1; ti, p=15, r=10; ta, p=18, r=20, v=6.

Coxae: yellowish-brown ventrally, IV wider than rest, I longer than rest.

Claws: all legs with three claws, paired claw with single tooth. Palp with single claw bearing single unequal tooth. Claws of leg IV longer than rest, and of leg I & II equal, claw of leg III smallest. Claw tufts absent.

Abdomen (Fig. 28): glossy blackish brown with silvery golden spike-like hairs in life; in alcohol, greyish-brown dorsally; covered with short and long hairs; ventrally yellowish covered with black hairs.

Spinnerets (Fig. 33): PMS digitiform; PLS, apical segment dome-shape. Covered with brown hair and spigots.

Spermathecae (Fig. 34): a pair of spermathecae, emerging from distal ends of each leaf-like sclerotized structure fused at base. Stalk on leaf-like structure supports stalk resembling an inverted bell.

Natural history.—The type specimens were collected near a small hamlet on the periphery of Aarey Milk Colony from degraded and barren flat land (Fig. 35). The surrounding area is dominated by Butea monosperma, which has been cleared at the collection site for agricultural purposes. All three specimens were collected within an area of less than 10 m². The burrows were mostly found near the base of dead B. monosperma or near large boulders. They were vertical, with a slant of 20-30° to the surface. The burrows were lined with a thick layer of silk, as seen in other species of the family Idiopidae and Ctenizidae (Z.A. Mirza & R.V. Sanap personal observation). The burrows in this particular area were constructed in a patch where the soil was dry and very difficult to dig, as compared to the loose and clay-rich soil preferred by I. bombayensis. All the burrows of the new species were vertical in orientation, but otherwise were constructed similarly to those of *I. bombayensis*. In less than 1 m², a total of six to eight large burrows were found, indicating high density. The collection site receives direct sunlight throughout the day during summer, but is covered with dense undergrowth throughout the monsoons up to late winter. The diameter of the entrance of the burrow of the male holotype was 12.5 mm and that of the female paratype was 14 mm; door thickness in the center of the male's burrow was 3.2 mm and that of the female was 2.7 mm; the door diameters of the male

and female burrows were 16.8 mm and 19.9 mm, respectively. An unhatched egg sac of this species was found at the base of the burrow where the types were collected on 4 July 2010, but a female was not present in the burrow. Careful searching revealed another burrow in the vicinity (ca. 100 mm away), which was occupied by a large female. This species is under threat, at least at the type locality, as it is only known from a small patch at the locality. The area in this region is degraded and used by locals for agricultural purpose and bootlegging, for which the forest is cleared, which adversely loosens the soil. The loose soil is washed away by the overflowing Vihar Lake and the seasonal forest streams, which leads to heavy destruction of the habitat of this and several other species. This species has also been seen in Sanjay Gandhi National Park (Fig. 40).

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New species of mite harvestmen from the Wet Tropics of Queensland, Australia, with commentary on biogeography of the genus *Austropurcellia* (Opiliones: Cyphophthalmi: Pettalidae)

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Abstract. Cyphophthalmi, commonly known as mite harvestmen, are a suborder of cryptic Opiliones with a global distribution. The genus Austropurcellia Juberthie 1988 is a lineage of mite harvestmen currently known from a small number of localities in the forests of Queensland, Australia. We describe four new species of Austropurcellia (A. alata, A. culutinis, A. despectata, and A. vicina) from museum lots; each new species is known from only a single collection and few specimens. We present the first key to the species of Austropurcellia, a catalogue of known collecting localities, and a distribution map. Although our current knowledge of the diversity and distribution of this genus is certainly incomplete, it is clear that these narrow-range endemics have great potential as a system for understanding the role of historical forest fragmentation in the evolution of rainforest animals.

Keywords: Biodiversity, dispersal, endemism, evolution

Australia's Wet Tropies World Heritage Area comprises 8,940 km² of tropical rainforests stretching from Townsville to Cooktown along Queensland's coast (Fig. 1), with the majority of land protected in national parks or other reserves. Although the area represents only 0.1% of the land area of Australia, it is home to a huge diversity of the continent's animal life, including a third of all Australian mammals and at least 75 regionally endemie vertebrates (Nix 1991). Over the past decade, interest in the fauna of the Wet Tropics has increased as the area has emerged as a model system for understanding patterns and processes of rainforest animal diversification (e.g., Joseph et al. 1995; Hugall et al. 2002; Hugall et al. 2003; Bell et al. 2004; Bell et al. 2007; Moussalli et al. 2009).

Cyphophthalmi, the arachnids commonly known as mite harvestmen, are a globally distributed suborder of Opiliones (harvestmen or daddy long-legs) that currently includes 185 described species and subspecies, including the four new species described here (Giribet 2011). They are small (2–5 mm long), morphologically conserved animals that spend their entire life cycle in leaf litter habitats, with the exception of one cave-dwelling species (Juberthie 1970). Mite harvestmen are short-range endemics (sensu Harvey 2002), with most species only known from a handful of localities within a 100-km radius, even in areas that have been sampled on a very fine geographic scale (e.g., Boyer & Giribet 2009). In addition, no Cyphophthalmi are known from any Darwinian islands (sensu Gillespie and Roderick 2002), such as islands formed de novo by volcanoes in the mid-ocean, suggesting that these invertebrates are unable to disperse across oceanic barriers (Giribet 2000) (but see Clouse and Giribet (2007) for a possible exception). Despite their limited vagility, these animals are found in leaf litter habitats worldwide. This paradox is explained by the great age of the Cyphophthalmi lineage. Their sister group is known from the Devonian (Dunlop et al. 2003), and recent molecular analyses have dated the origin of the suborder at 345 Ma (Giribet et al. 2009). These animals also have excellent persistence; specifically, they require only very small patches of suitable habitat and therefore can withstand severe habitat contraction. For example, one of the authors (SLB) has collected these animals in New Zealand from a patch of forest habitat measuring only 10 m at its widest and surrounded on all sides by pasture grazed by cattle. Because these animals display species-level endemism on a fine geographic scale, their biodiversity is likely poorly known in most areas, with exceptions in places such as New Zealand where they have been studied with dense geographic sampling (e.g., Boyer & Giribet 2003; Boyer et al. 2007; Boyer & Giribet 2009).

The cyphophthalmid family Pettalidae has a classical Temperate Gondwanan distribution, with representatives in Chile, South Africa, Madagascar, Sri Lanka, New Zealand and Australia. Pettalidae is a monophyletic group, with estimated ages of nodes within the phylogeny consistent with the hypothesis that the distribution of the family predates the breakup of the former supercontinent (Boyer & Giribet 2007; Boyer et al. 2007; Giribet et al. 2009). Members of the pettalid genus *Austropurcellia* are known from a handful of localities scattered throughout the Wet Tropics, but previous authors have suggested that the diversity of the Queensland lineage is poorly known (Juberthie 1988; Giribet 2003).

We performed a survey of collections of mite harvestmen from Queensland, including collections from the Queensland Museum, the Australian National Insect Collection, the Western Australian Museum, and the Muséum d'Histoire Naturelle, Geneva. As a result, we have identified four new morphologically distinct species of *Austropurcellia* from the Wet Tropics, bringing the total number of known species to ten and confirming expectations that there is significant undeseribed diversity within this genus. Here, we deseribe these new species and discuss the biogeography of *Austropurcellia*.

METHODS

Localities were mapped with ArcInfo 10 (ESRI, Incorporated) using recorded collection coordinates when available, or by estimation when coordinates were not available.

For each new species, we examined one male specimen with a JEOL JSM-6610LV Scanning Electron Microscope (SEM). Total body length refers to the distance between the anterior median and posterior median margins of the dorsal scutum.

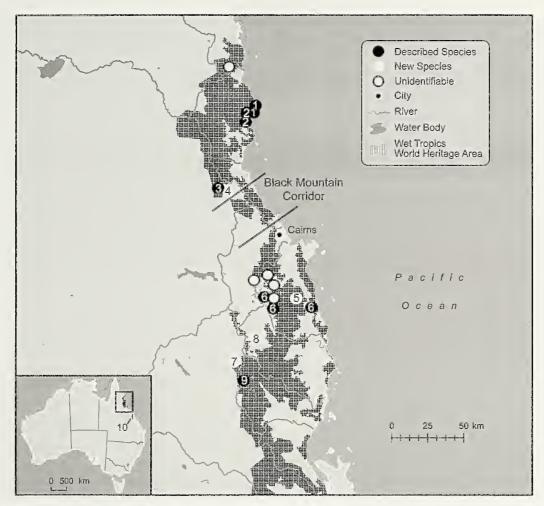


Figure 1.—Distribution of the genus Austropurcellia. Black points indicate previously described species; white points indicate new species. 1. A. forsteri. 2. A. arcticosa. 3. A. scoparia. 4. A. vicina n. sp. 5. A. culminis n. sp. 6. A. daviesae. 7. A. alata n. sp. 8. A. despectata n. sp. 9. A. woodwardi. 10. A. capricornia.

Lengths of leg and palp articles were measured on their dorsal side, from anterior to posterior margin, along the mid-line; widths (depths) on the lateral side, at the widest point, except for tarsus IV of the male, which was measured at the distal point of insertion of the adenostyle. Tarsal length does not include the claw. All appendage measurements refer to the paratype specimens studied with SEM with the exception of Austropurcellia alata, for which the mounted specimen is designated the holotype. We took light microscope images of holotype animals using an Olympus SZX10 microscope.

The specimens utilized in this study are lodged in the following institutions: Australian National Insect Collection, CSIRO, Canberra, Australia (ANIC); Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA (MCZ); Muséum d'Histoire Naturelle, Geneva, Switzerland (MHNG); Muséum National d'Histoire Naturelle, Paris, France (MNHN); and Queensland Museum, Brisbane, Australia (QM).

RESULTS AND DISCUSSION

Diversity and taxonomy of Austropurcellia.—In his 1988 description of the genus Austropurcellia, C. Juberthie states that he received a number of cyphophthalmid specimens from Valerie Todd Davies, then Curator of Arachnids at the Queensland Museum that (in addition to A. scoparia) included

eight new species from the genus Neopurcellia and two new species of Rakaia. Juberthie (1998, 2000) described two more of these, Rakaia daviesae Juberthie 1988 and Neopurcellia forsteri Juberthie 2000, both of which have subsequently been transferred to Austropurcellia (Boyer and Giribet 2007). Although the specimens representing the remaining undescribed species cannot be located at the present time (G. Giribet personal communication), Juberthie's remark indicates that the diversity of Cyphophthalmi from Queensland was not well described in 1988. The present study, based on specimens from museum lots borrowed from ANIC, QM and MHNG, adds four more species, but mapping of known localities strongly suggests that Austropurcellia's diversity remains poorly sampled.

Morphological diversity of Austropurcellia is found in the male anal plate and associated scopulae, male tarsus IV (which varies both in the degree of bisegmentation and the shape of the adenostyle) and the degree of ornamentation of tarsi and metatarsi of legs I and II. In recent years, our understanding of the characters that distinguish major lineages within the family Pettalidae has been altered by molecular phylogenetic studies. For example, the character once used to distinguish Neopurcellia from Rakaia, bisegmentation of tarsus IV of the male, is more rapidly evolving than previously suspected. Molecular phylogenies have demonstrated that tarsus IV has

switched between bisegmented, entire, and partially bisegmented several times over the course of the evolution of the family Pettalidae (Boyer & Giribet 2007, 2009). The discovery of Austropurcellia culminis, which differs from A. daviesae only in degree of bisegmentation of tarsus IV, confirms that this character is rapidly evolving. Austropurcellia culminis and A. daviesae (Juberthie 1989) both occur around Bellenden Ker, which taken together with their extremely similar morphology suggests that these taxa are sister species. The minimal locality data currently available suggests that these species may occur at different elevations; while A. daviesae is known from several localities, A. culminis has only been collected at the summit of Bellenden Ker itself. Genetic data collected from many individuals from several localities, as well as precisely georeferenced locality data, would help to clarify the evolutionary relationships of these two very similar (but morphologically distinct) species.

Another character that was formerly considered to be of taxonomic importance at the level of genus is the extent of ornamentation of the first and second metatarsi. Juberthie (1988) originally diagnosed the genus Austropurcellia as distinguished from Rakaia by the presence of a bisegmented fourth tarsus of the male, and from Neopurcellia by the degree of ornamentation of the second metatarsus (fully ornamented in Austropurcellia, ornamented only in the basal half in Neopurcellia). Our work confirms that the degree and type of ornamentation found on metatarsus II varies within Austropurcellia; however, rather than characterizing metatarsus II as fully ornamented in some and partially ornamented in others, we distinguish between uniformly ornamented versus nonuniformly ornamented. In some specimens with a nonuniformly ornamented metatarsus I and II, this character is manifested as an abrupt break between ornamentation types, with distinctly ornamented and unornamented halves of the metatarsus separated by a demarcation resembling a suture (Fig. 13E). In other specimens, the shift is more subtle, with the distal half of the metatarsus bearing significant ornamentation that is nonetheless reduced in density with respect to the proximal half of the metatarsus. Hence, Boyer and Giribet (2007) coded the metatarsus II as fully ornamented in A. daviesae and A. forsteri (Juberthie 2000), species that we here consider non-uniformly ornamented.

Biogeography of *Austropurcellia*.—Like other Cyphophthalmi, *Austropurcellia* species display endemism on a very fine geographic scale, with each species currently known from just a small handful of localities all within 50 km of each other (Fig. 1). Therefore, we expect that exploration of forested areas throughout the Wet Tropics of Queensland will yield many new collecting sites and additional new species. For example, these animals have been collected in Daintree National Park at Cooper Creek and Emmagen Creek, but nowhere else within the 1200 km² of the park's area. Similarly, there are no known collecting localities within Girringun National Park or Paluma Range National Park. Species distribution modeling (also known as environmental or ecological niche modeling) could be used to make predictions about areas that would hold most promise for "prospecting" for new *Austropurcellia* localities.

The Wet Tropics represent the largest remnant of the Gondwana-derived rainforests that once dominated the entire continent of Australia, but declined throughout the Tertiary

(Adam 1992; BMR Paleogeographic Group 1990; Harrison & Dodson 1993; Nix 1991; Truswell 1993). Today the Wet Tropics is comprised of isolated "islands" of montane rainforest surrounded by warmer or more xeric habitats. The history of the area during the Last Glacial Maximum through the present has been well studied by researchers working within several different disciplines. Palynological, phylogeographic, and biogeographic data all indicate that much of the Quaternary rainforest in this region was severely contracted, confined to two isolated areas each of which contained many small refugia (Nix 1991; Kershaw 1994; Joseph et al. 1995; Schneider et al. 1998; Hugall et al. 2002, 2003). These findings are consistent with results from paleoclimate modeling, which predict that temperature and moisture regimes suitable for rainforest growth would have been fragmented and disjunct throughout the region during the Last Glacial Maximum (LGM) (Nix 1991; Graham et al. 2006). During the transition from cool-dry to cool-wet conditions, commencing approximately 8000 years ago, rapid expansion of the rainforest occurred, followed by another less severe contraction to produce the current distribution of forest habitat (Kershaw 1984; BMR Palaeogeographic Group 1990; Hopkins et al. 1993).

In vertebrate groups, this recent history of climate change has resulted in a pattern of strong population divergence between "islands" of rainforest habitat (Joseph et al. 1995; Schneider and Moritz 1999; Schneider et al. 1999; Phillips et al. 2004). In particular, major genetic divergences coincide geographically with a feature known as the Black Mountain Corridor (BMC) (Fig. 1), a large dry area separating the Carbine and southern Atherton Tablelands 18,000 years ago that is predicted by paleoclimate modeling (Winter 1984; Nix 1991; Hugall et al. 2002; Graham et al. 2006).

It is predicted that small, slow-dispersing invertebrate taxa should display phylogenetic and/or phylogeographic structure at a finer geographic scale than more vagile vertebrates (Moritz et al. 2001). A limited number of published studies of Wet Tropics fauna support this hypothesis. The work of Bell at al. (2004, 2007) on the Wet Tropics endemic dung beetle genus Temnoplectron demonstrated that barriers between species coincide geographically with phylogeographic breaks found in vertebrates, and are likely explained by habitat fragmentation during the LGM. A landmark study by Hugall et al. (2002) investigated the phylogeographic relationships of the terrestrial gastropod Gnarosophia bellendenkerensis, which is endemic to the Wet Tropics rainforests, using a spatially explicit approach incorporating paleoclimate modeling. They found that although there was a major genetic break associated with the BMC, it was one of only several deep divisions within the species (Hugall et al. 2002; Hugall et al. 2003).

Within Austropurcellia, the BMC marks a split not between populations or species, but rather between groups of species – though whether those groups represent evolutionary lineages is currently unknown. Within the Wet Tropics, four morphologically distinct species (A. arcticosa (Cantrell 1980), A. forsteri (Juberthie 2000), A. scoparia Juberthie 1988, and A. vicina) occur north of the BMC, and five occur south of the BMC (A. alata, A. culminis, A. daviesae (Juberthie 1989), A. despecata, and A. woodwardi (Forster 1955)) (Fig. 1), and there are almost certainly many more related species awaiting discovery, especially south of the BMC. Beyond the Wet Tropics, the

only described species of Austropurcellia is A. capricornia (Todd Davies 1977) (Fig. 1). At present, phylogenetic analysis of the family Pettalidae has not resolved relationships within Austropurcellia (Boyer & Giribet 2007, 2009). The addition of more individuals, more populations and more species to a molecular phylogenetic analysis of the genus could result in a robust evolutionary tree that would permit tests of hypotheses about the role of forest fragmentation during the LGM in driving the diversification of these cryptic dispersal-limited arachnids. In addition to a phylogeny, time calibration is essential in such tests. A recent phylogeny of the Opiliones calibrated with time points based on fossil harvestman concluded that the genus Austropurcellia is 102 Ma old, with a standard deviation of 16 Ma (Giribet et al. 2009). Given the great age of the lineage, and the ability of these animals to persist in tiny patches of appropriate habitat, the forest refugia of the LGM may have acted as museums rather than cradles of biodiversity in the case of the mite harvestmen.

TAXONOMY

Family Pettalidae Genus *Austropurcellia* Juberthie 1988

Austropurcellia Juberthie 1988:133, Boyer & Giribet 2007:347.

Type species.—Austropurcellia scoparia Juberthie 1988, by original designation.

Diagnosis.—We use the generic diagnosis formulated by Boyer & Giribet (2007), with one modification. Although those authors state that scopulae are absent from tergite VIII, Todd Davies' (1977) description of A. capricornia does depict scopulae emerging from tergite VIII. Ozophores in dorsal 45° position. Eyes present, incorporated into ozophores, without

lenses. No projections surrounding gonostome. Male exocrine glands may be present in anal region. Scopulae present on anal plate. Tergite IX divided. Robust ventral process on the proximal article of the chelicerae absent; prominent apodeme on the distal article of chelicerae. Prominent ventral process on trochanter of palp. Solea in tarsus I. Male tarsus IV bisegmented dorsally to fully bisegmented. Adenostyle extremely robust, with height no more than twice base length.

Taxonomic history.—The first mite harvestman discovered in Australia, Rakaia woodwardi, was described from the Wet Tropics by Forster (1955). Two additional species from Queensland, Neopurcellia capricornia Todd Davies 1977 and Rakaia arcticosa Cantrell 1980, were described more than two decades later, and Juberthie (1989, 2000) subsequently added R. daviesae Juberthie 1989 and N. forsteri Juberthie 2000. Juberthie (1988) also described the monotypic genus Austropurcellia (type species scoparia) from Queensland. The genera Rakaia and Neopurcellia as originally described also included species from New Zealand, but recent phylogenetic analyses have demonstrated that the Queensland species comprise a monophyletic group and are more closely related to each other than they are to New Zealand taxa. As a result, all Queensland Rakaia and Neopurcellia have been transferred to Austropurcellia by Boyer and Giribet (2007), who also re-evaluated all morphological characters previously thought to be of taxonomic significance and developed a new diagnosis for the genus.

Species account and distribution.—Ten valid species (including the four new species described here) are known, all occurring in Queensland, Australia. Nine of the ten occur within the Australian Wet Tropics, with *A. capricornia* known from further south at Finch Hatton, near Mackay (Fig. 1).

KEY TO THE KNOWN SPECIES OF AUSTROPURCELLIA

1.	Male tarsus IV fully divided
	Male tarsus IV partially divided
2.	Triangular projections emerging laterally from tergite VIII
	Tergite VIII without lateral projections
3.	Scopulae emerging from tergite VIII
	Tergite VIII without scopulae
4.	Scopula emerging from anterior margin of anal plate
	Anterior margin of anal plate without scopula
5.	Twisted scopula emerging from center of anal plate
	Center of anal plate without scopula
6.	Anal plate bilobed posteriorially with central posterior scopula
7.	Long scopula emerging from posterior margin of anal plate to cover part of tergite VIII
	Small scopula not extending past anal plate
8.	Anal plate without distended protrustions on anterior margin
	Anal plate with distended protrustions on anterior margin
9.	Long scopula emerging from posterior margin of anal plate to cover part of tergite VIII
	Small scopula not extending past posterior margin of the anal plate
10.	Thick median scopula
	Small twisted central scopula

Austropurcellia alata new species Figs. 2-5

Type.—Holotype male, 20 km S of Ravenshoe, Queensland, Australia, 1900 m, 17°49S,145°32′E, 3 July 1971, Taylor and Feehan, ANIC berlesate 358 (ANIC).

Other material examined.—The only known specimen beyond the holotype is the one individual photographed during this study (Figs. 2A–C), which was subsequently lost inside equipment during environmental SEM.

Etymology.—Latin adjective: alata = winged. The specific epithet refers to the unusual lateral projections of tergite VIII.







Figure 2.—Austropurcellia alata n. sp., male holotype: A. Dorsal view; B. Ventral view; C. Lateral view. Scale bars = $500 \mu m$.

Diagnosis.—Pettalid with dorsal scutum flat (Figs 2A, 3A). Trochanter of palp with conspicuous ventral process (Fig. 5A). Scopulae originating from center of anal plate, but not observed in either specimen examined (Figs. 3B, 4C). Prominent lateral projections emerging from tergite VIII

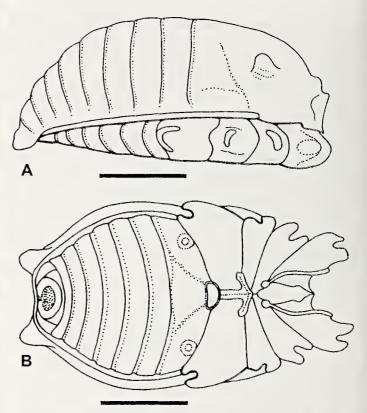


Figure 3.—Austropurcellia alata n. sp., male holotype: A. Lateral view; B. Ventral view. Scale bars $= 500 \mu m$.

(Figs. 3B, 4C). Tarsus bisegmented with triangular adenostyle (Fig. 5G).

Description.—Total length of male holotype 2.24, greatest width 1.27 mm. Body brown-yellow (in alcohol), with most of the dorsal surface and legs showing a tuberculate-granulate microstructure (Figs. 4A, B). Anterior margin of dorsal scutum with unusual prominent posterio-lateral projections; prosomal region trapezoidal (Figs. 3B, 4A, B). Ozophores conical positioned at a 45° angle (Fig. 4A). Transverse opisthosomal sulci distinct by lacking granulation (Fig. 4A). Longitudinal opisthosomal sulcus present in posterior-most region of the animal (Fig. 4A). Dorsal scutum flat; opisthosomal region reaching its maximum width at segment II (Figs. 3B, 4A, B)

Coxae of legs I and II mobile; coxae of remaining legs fixed. Ventral prosomal complex of male with coxae II–IV meeting in the midline (Figs. 3B, 4B), Male gonostome small, subtriangular, wider than long, bordered on posterior margin by the first opisthosomal sternite; male gonostome shorter than length of seam of contact of left and right coxae IV parallel to midline (Fig. 4D). No female specimens available.

Spiracles C-shaped (sensu Giribet and Boyer 2002), with both edges recurving internally as found in the "open circle" type (Fig. 4E). Sternal opisthosomal region without modifications or glandular pores (Fig. 4D). Anal region with sternites 8 and 9 and tergite IX free, not forming a corona analis (Figs. 3B, 4C). Area of contact of tergite IX and sternite 9 of "pettalid" type (Giribet and Boyer 2002), in which tergite IX laterally covers sternite 9 and clearly meets sternite 8 (Figs. 3B, 4C). Anal plate of male with central scopula apparently present but broken off in both male specimens

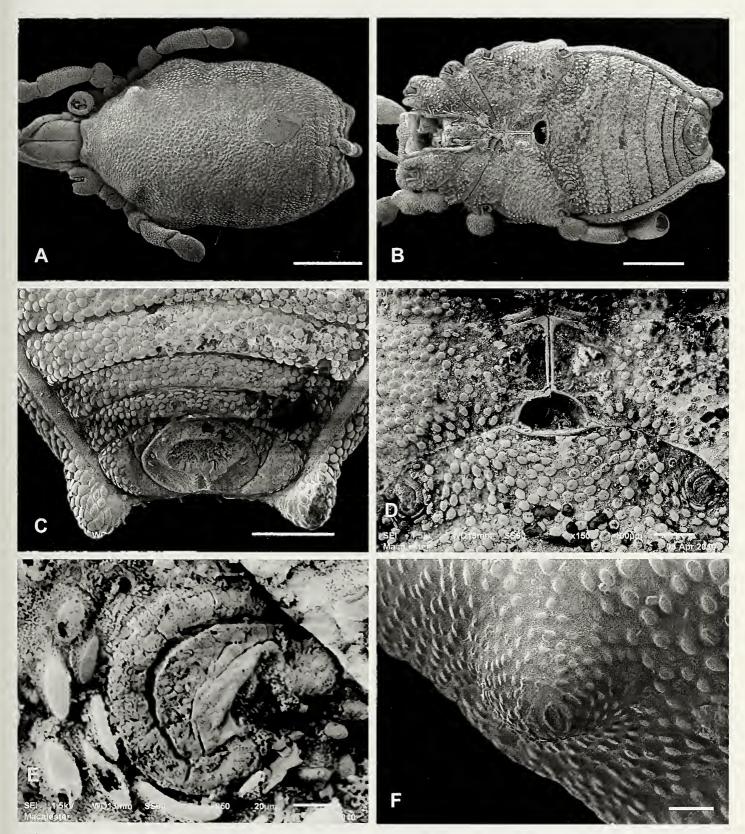


Figure 4.—Austropurcellia alata n. sp: A. Male paratype, dorsal view, scale bar = $500 \,\mu m$; B. Male holotype, ventral view, scale bar = $500 \,\mu m$; C. Male holotype, posterior ventral view, scale bar = $200 \,\mu m$; D. Male holotype, gonostome and sternal area, scale bar = $100 \,\mu m$; E. Male holotype, spiracle, scale bar = $20 \,\mu m$; F. Male paratype ozophore, scale bar = $50 \,\mu m$.

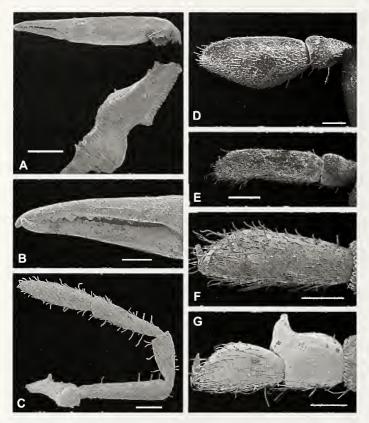


Figure 5.—Austropurcellia alata n. sp., male holotype: A. Chelicera, scale bar = 200 μ m; B. Detail of chelicerae, scale bar = 200 μ m; C. Palp, scale bar = 100 μ m; D. Tarsus I; E. Tarsus II; F. Tarsus III. G. Tarsus IV; scale bar = 100 μ m for all tarsi.

examined (Fig. 4C). Cuticle with granulate surface in all ventral areas except for anal plate (Figs. 4A–C). No anal glandular pores visible.

Chelicerae short and extremely robust. Proximal article of chelicerae with dorsal crest ["dorsal ridge" of Hansen and Sorensen (1904) and Forster (1948); "dorsal transverse crest" of Juberthie (1970)], without ventral process (Fig. 5A). Two types of dentition, as is typical in pettalids (Fig. 5B).

Palp with a prominent ventral process on the trochanter (Fig. 5C).

Surface of most articles clearly ornamented with granules; all tarsi smooth (Figs. 5F, G) and metatarsi I and II partially ornamented (Figs. 5D, E). Ventral side of tarsus I with solea (Fig. 5D). Tarsus IV of the male bisegmented, bearing a large thick adenostyle projecting upward and slightly distally (Fig. 5G). Measurements from holotype male of leg articles from proximal to distal (given in µm): leg I – 218, 554, 212, 417, 172, 443; leg II – 175, 440, 174, 352, 112, 375; leg III – 179, 356, 167, 334, 152, 315; leg IV – 311, 505, 303, 390, 165, 423.

Remarks.—Austropurcellia alata is known only from the type locality in the Atherton Tableland of north-eastern Queensland.

Austropurcellia culminis new species Figs. 6–9

Types.—Holotype male, Bellenden Ker Summit, 1500 m, 40 km SSE of Cairns, Queensland, Australia, 17°14′S,145°51′E,

20–22 January 1992, I.D. Burekhardt (QM 90599). Paratypes: 5 males, 8 females, 1 juvenile, collected with holotype (MHNG).

Etymology.—Latin noun: culminis = of the summit. The specific epithet refers to the type locality of the species, the summit of Bellenden Ker. This species is very similar to A. daviesae, which is found at several lower-altitude localities.

Diagnosis.—Pettalid with dorsal scutum flat (Figs. 6A, 7A). Trochanter of palp with conspicuous ventral process (Figs. 9C, D); chelicerae with robust apodeme (Fig. 9A); tarsus I with distinct solea (Fig. 9E). Scopulae emerging from center of anal plate (Fig. 8C). Male tarsus IV smooth and fully bisegmented with thick triangular adenostyle (Fig. 9H).

Description.—Total length of male holotype 1.5 mm, greatest width 0.8 mm. Body brown-yellow (in alcohol), with most of the dorsal surface and legs showing a tuberculate-granulate microstructure (Figs. 8A, B). Anterior margin of dorsal scutum without projections. Ozophores conical positioned at a 45° angle. Transverse opisthosomal sulci distinct by lacking granulation; longitudinal sulcus absent (Fig. 8A). Dorsal scutum flat; opisthosomal region reaching its maximum width at segment II (Figs. 7B, C, 8A, B).

Coxae of legs I and II mobile; coxae of remaining legs fixed. Ventral prosomal complex of male with coxae II–IV meeting in the midline (Fig. 7B). Male gonostome small, subtriangular, wider than long, bordered on posterior margin by the first opisthosomal sternite; male gonostome shorter than length of seam of contact of left and right coxae IV parallel to midline (Fig. 8D). Ventral prosomal complex of female with only coxae II meeting at the midline. Female gonostome roughly round in shape, with the edges of coxae of leg IV and first opisthosomal sternite forming a partial "tube" at the posterior margin of the opening.

Spiracles C-shaped (sensu Giribet and Boyer 2002) (Fig. 8E). Sternal opisthosomal region without modifications or glandular pores. Anal region with a partial corona analis: sternites 8 and 9 and tergite IX fused, although tergite IX is bisegmented. Anal region with sternites 8 and 9 and tergite IX free, not forming a corona analis. Area of eontact of tergite IX and sternite 9 of "pettalid" type (Giribet and Boyer 2002) in which tergite IX laterally covers sternite 9 and clearly meets sternite 8. Anal plate of male with small central scopula (Fig. 8C). Cuticle with granulate surface in all ventral areas (Figs. 8A, B). Anal glandular pore not visible.

Chelicerae short and extremely robust (Fig. 9A). Proximal article of chelicerae with dorsal crest ("dorsal ridge" of Hansen and Sørensen (1904) and Forster (1948); "dorsal transverse crest" of Juberthie (1970)), without ventral process (Fig. 9A). Distal article of chelicerae with a conspicuous apodeme (Fig. 9A). Two types of dentition on mobile article of chelicerae, as is typical of pettalids (Fig. 9B).

Palp with a prominent ventral process on the trochanter (Fig. 9C, D).

Legs with all claws smooth, without ventral dentition or lateral pegs. In legs I and II the metatarsus is partially ornamented and the tarsus is smooth, while in legs III and IV the metatarsus is fully ornamented and the tarsus is smooth (Figs. 9E–H). Ventral side of tarsus I with solea (Fig. 9E). Tarsus IV of the male fully bisegmented, bearing a large thick adenostyle (Fig. 9H). Tarsus IV of the female without modifications. Measurements from holotype male of leg

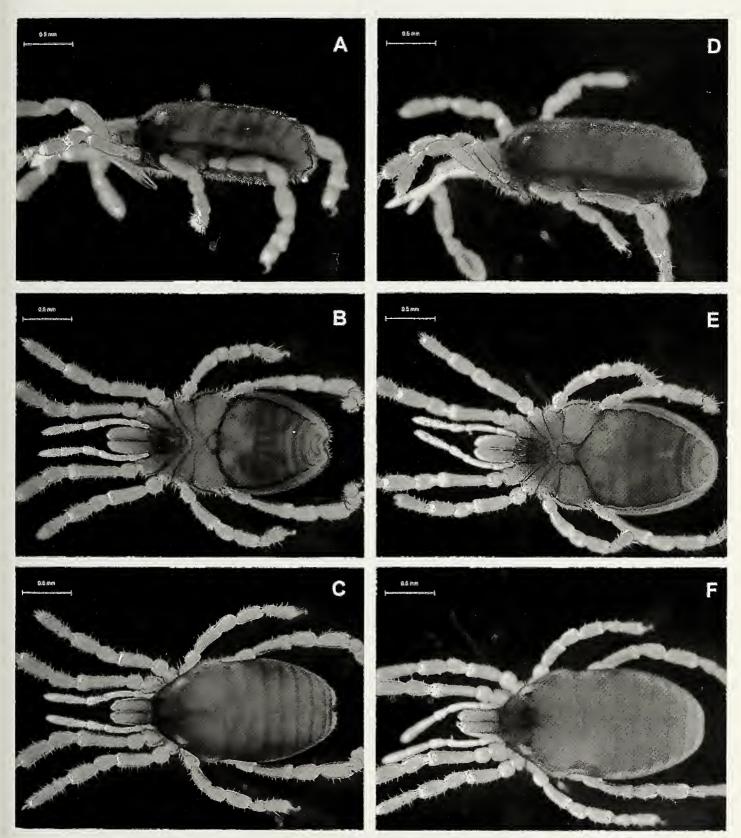


Figure 6.—Austropurcellia culminis n. sp., male holotype and female paratype: A. Male, lateral view; B. Male, ventral view; C. Male, dorsal view; D. Female, lateral view; E. Female, ventral view; F. Female, dorsal view. Scale bars = 500 µm.

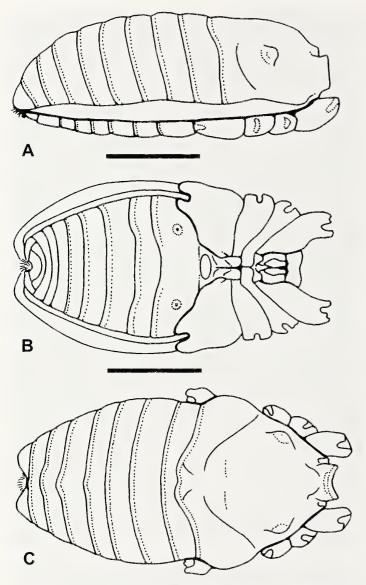


Figure 7.—Austropurcellia culminis n. sp., male holotype: A. Lateral view; B. Ventral view; C. Dorsal view. Scale bars = 500 µm.

articles from proximal to distal (given in μ m): leg I - 149, 478, 234, 364, 161, 372; leg II - 128, 380, 201, 266, 138, 334; leg III - 131, 308, 140, 252, 136, 295; leg IV - 280, 341, 197, 328, 138, 342.

Remarks.—Austropurcellia culniuis is known only from the type locality at the summit of Bellenden Ker.

Austropurcellia despectata new species Figs. 10–13

Types.—Holotype male, from Millaa Millaa, Queensland, Australia,17°31′S, 145°37′E (estimated), 15 May 1990, I.D. Naumann, J.C. Cardale, ANIC berlesate 674 (ANIC). Paratypes: 1 adult male, 1 subadult male 1 juvenile, collected with holotype (ANIC).

Etymology.—Latin adjective: despectata = touristed, viewed, observed. The specific epithet refers to the much-visited and oft-photographed waterfalls for which Millaa Millaa, the type locality for the species, is famous.

Diagnosis.—Pettalid with dorsal scutum flat (Figs. 10A, 11A). Trochanter of palp with conspicuous ventral process (Fig. 13D); chelicerae with robust apodeme (Fig. 13A). Anal

plate with small central scopula and two smooth anterior protrusions easily visible in lateral view (Figs. 10A, 11A, 12C). Metatarsi I and II with non-ornamentation that is lighter distally than proximally, and tarsi I and II lightly ornamented in basal-most area (Figs. 13E–H). Tarsus IV with light ornamentation in basal-most area, and partially bisegmented with thick triangular adenostyle (Fig. 13H).

Description.—Total length of male holotype 1.9 mm, greatest width 1.3 mm. Body brown-yellow (in alcohol), with most of the dorsal surface and legs showing a tuberculate-granulate microstructure (Figs. 12A, B). Anterior margin of dorsal scutum without projections. Ozophores conical positioned at a 45° angle. Transverse and longitudinal opisthosomal sulci distinct by lacking granulation (Fig. 12A). Dorsal scutum flat; opisthosomal region reaching its maximum width at segment II (Fig. 11B, C, 12A, B).

Coxae of legs I and II mobile; coxae of remaining legs fixed. Ventral prosomal complex of male with coxae II, III, and IV meeting in the midline (Figs. 11B, 12B), Male gonostome small, subtriangular, wider than long, bordered on posterior margin by the first opisthosomal sternite; male gonostome shorter than length of seam of contact of left and right coxae IV parallel to midline (Fig. 12D). No female specimens available for examination.

Spiracles C-shaped (sensu Giribet and Boyer 2002) (Fig. 12E). Sternal opisthosomal region without modifications or glandular pores. Anal region with sternites 8 and 9 and tergite IX free, not forming a corona analis (Fig. 12C). Area of contact of tergite IX and sternite 9 of "pettalid" type (Giribet and Boyer 2002) in which tergite IX laterally covers sternite 9 and clearly meets sternite 8 (Fig. 12C). Anal plate of male with central scopula and two distinctive ventral swellings anterior to scopula (Fig. 12C). Cuticle with granulate surface in all ventral areas except for anal plate (Fig. 12C). Anal glandular pore located at junction of tergites VIII, IX, and anal plate (Fig. 12C).

Chelicerae short and extremely robust (Fig. 13A). Proximal article of chelicerae with dorsal crest ["dorsal ridge" of Hansen and Sørensen (1904) and Forster (1948); "dorsal transverse erest" of Juberthie (1970)], without ventral process (Fig. 13A). Distal article of chelicerae with a conspicuous apodeme (Fig. 13A). Two types of dentition, as is typical of pettalids (Fig. 13B).

Palp with a prominent ventral process on the trochanter (Fig. 13D).

Legs with all claws smooth, without ventral dentition or lateral pegs. Metatarsus I fully ornamented and tarsus I lightly ornamented in the basal-most area; metatarsus II partially ornamented and tarsus II smooth; metatarsus III completely ornamented and tarsus III smooth; metatarsus completely ornamented and tarsus IV lightly ornamented in basal-most area (Figs. 13E). Tarsus IV of the male partially bisegmented, bearing a large thick adenostyle projecting upward (Fig. 13H). Measurements from holotype male of leg articles from proximal to distal (given in μm): leg I – 167, 480, 214, 323, 138, 362; leg II – 155, 389, 166, 287, 130, 323; leg III – 160, 328, 169, 260, 129, 311; leg IV – 220, 437, 220, 324, 137, 370.

Remarks.—Austropurcellia despectata is known only from the type locality in Millaa Millaa.

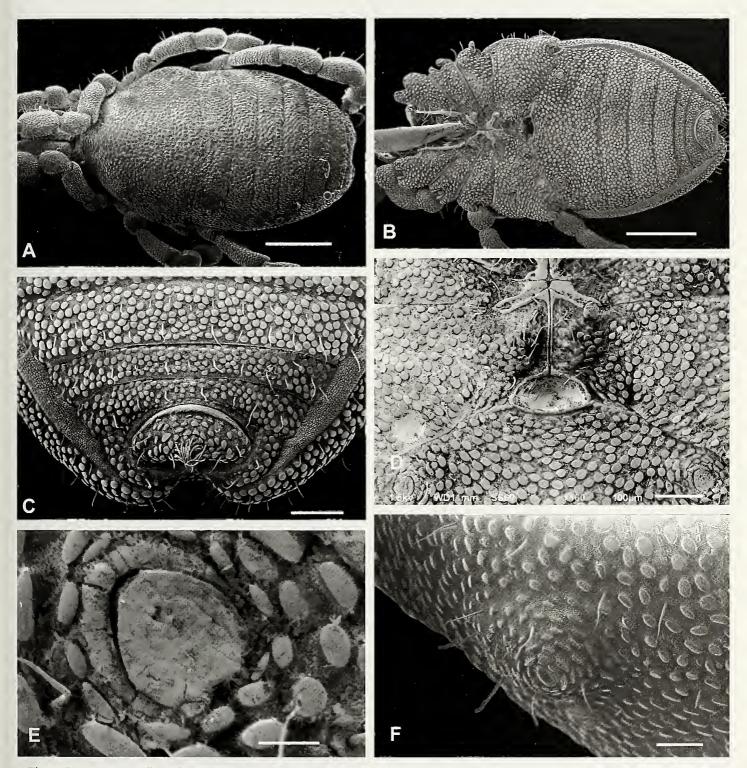


Figure 8.—Austropurcellia culminis n. sp., male paratype: A. Dorsal view, scale bar = $400 \mu m$; B. Ventral view, scale bar = $400 \mu m$; C. Posterior ventral region, scale bar = $100 \mu m$; D. Gonostome area, scale bar = $100 \mu m$; E. Spiracle, scale bar = $20 \mu m$; F. Ozophore, scale bar = $50 \mu m$.

Austropurcellia vicina new species Figs. 14–16

Types.—Holotype male, Mt. Lewis via Julatten, 16°34′S, 145°18′E (estimated), 21 May 1980, I.D. Naumann, J.C. Cardale; ANIC berlesate 679 (ANIC). Paratype: 1 male, collected with holotype (ANIC).

Etymology.—Latin noun, vicina = neighbor. The specific epithet refers to this species' proximity to the type locality for the genus, which is Julatten.

Diagnosis.—Pettalid with dorsal scutum flat (Figs. 14C, 15C). Trochanter of palp with conspicuous ventral process (Fig. 17B); chelicerae with robust apodeme (Fig. 17A), tarsus

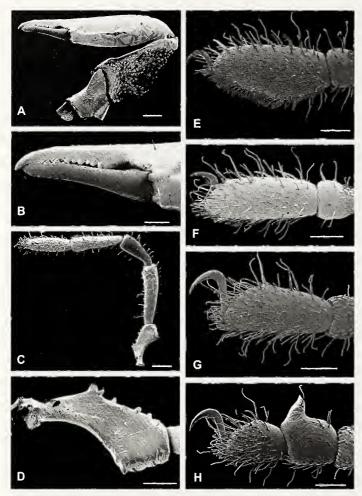


Figure 9.—Austropurcellia culminis n. sp., male paratype: A. Chelicera, scale bar = $100~\mu m$; B. Detail of chelicerae, scale bar = $50~\mu m$; C. Palp, scale bar = $100~\mu m$; D. Trochanter of palp, scale bar = $50~\mu m$; E. Tarsus I; F. Tarsus II. G. Tarsus III; H. Tarsus IV; scale bar = $100~\mu m$ for all tarsi.

I with distinct solea (Fig. 17C). Scopulae emerging from center of anal plate (Fig. 16B). Tarsus fully bisegmented with thick triangular adenostyle (Fig. 17F).

Description.—Total length of male paratype 1.27 mm at widest, 1.92 mm long. Body brown-yellow (in alcohol), with most of the dorsal surface and legs showing a tuberculate-granulate microstructure (Fig. 16A). Anterior margin of dorsal scutum without projections (Figs. 16A, B). Ozophores conical an positioned at a 45° angle. Transverse and longitudinal opisthosomal sulci distinct by lacking granulation. Dorsal scutum flat; opisthosomal region reaching its maximum width at segment II (Figs. 15B, C).

Coxae of legs I and II mobile; coxae of remaining legs fixed. Ventral prosomal complex of male with coxae II–IV meeting in the midline (Fig. 16D). Male gonostome small, subtriangular, wider than long, bordered on posterior margin by the first opisthosomal sternite; male gonostome shorter than length of seam of contact of left and right coxae IV parallel to midline (Fig. 16D).

Spiracles C-shaped (sensu Giribet and Boyer 2002) (Fig. 16C). Sternal opisthosomal region without modifications or glandular pores. Anal region without a corona analis, with tergite IX bisegmented. Area of contact of tergite IX and







Figure 10.—Austropurcellia despectata n. sp., male holotype: A. Lateral view; B. Ventral view; C. Dorsal view. Scale bars = 500 μm.

sternite 9 of the "pettalid" type (Giribet and Boyer 2002) in which tergite IX laterally covers sternite 9 and clearly meets sternite 8 (Fig. 16B). Anal plate of male with large, twisted central scopula (Fig. 16B). Cuticle with granulate surface in all

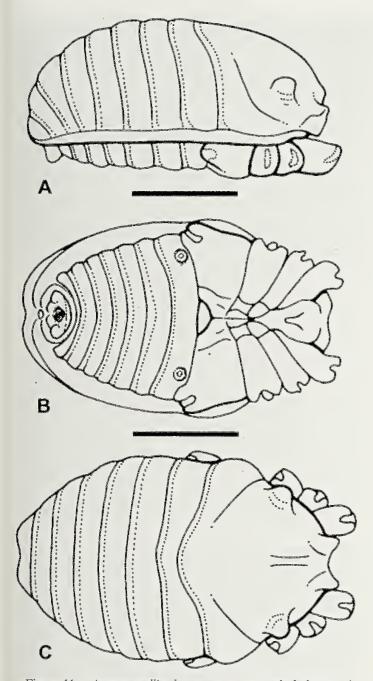


Figure 11.—Austropurcellia despectata. n. sp. male holotype: A. Lateral view; B. Ventral view; C. Dorsal view. Scale bars = $500 \mu m$.

ventral areas (Fig. 16A). Anal glandular pore not visible (Fig. 16B).

Chelicerae short and extremely robust (Fig. 17A). Proximal article of chelicerae with dorsal crest ["dorsal ridge" of Hansen and Sørensen (1904) and Forster (1948); "dorsal transverse crest of Juberthie (1970)], without ventral process (Fig. 17A). Distal article of chelicerae with a conspicuous apodeme (Fig. 17A). Two types of dentition on mobile article of chelicerae, as is typical of pettalids.

Palp with a prominent ventral process on the trochanter (Fig. 17B).

Legs with all claws smooth, without ventral dentition or lateral pegs. In legs I and II the metatarsus is partially ornamented and the tarsus is smooth, while in legs III and IV

the metatarsus is fully ornamented and the tarsus is smooth (Figs. 17C–F). Ventral side of tarsus I with solea (Fig. 17C). Tarsus IV of the male fully bisegmented, bearing a large thick adenostyle (Fig. 17F). Measurements from holotype male of leg articles from proximal to distal (given in μ m): leg I – 145, 560, 260, 387, 145, 456; leg II – 162, 420, 222, 301, 145, 389; leg III – 102, 405, 200, 291, 157, 351; leg IV – 253, 440, 250, 360, 131, 444.

Remarks.—Austropurcellia vicina is known only from the type locality at Mt. Lewis, near Julatten.

ACKNOWLEDGMENTS

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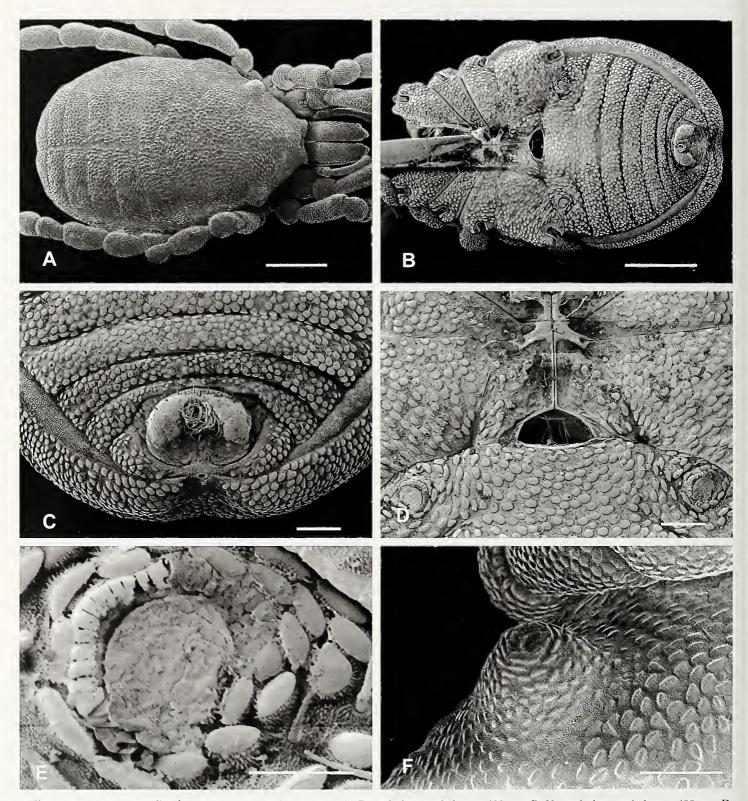


Figure 12.—Austropurcellia despectata n. sp., male paratype: A. Dorsal view, scale bar = $400 \, \mu m$; B. Ventral view, scale bar = $400 \, \mu m$; D. Posterior ventral region, scale bar = $100 \, \mu m$; D. Gonostome and sternal area, scale bar = $100 \, \mu m$; E. Spiracle, scale bar = $40 \, \mu m$; F. Ozophore, scale bar = $100 \, \mu m$.

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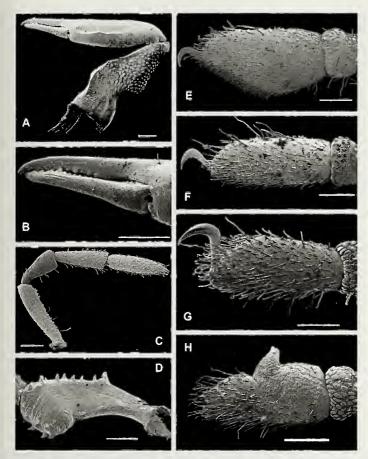


Figure 13.—Austropurcellia despectata n. sp., male paratype: A. Chelicera, seale bar = 100 $\mu m;$ B. Detail of chelicerae, scale bar = 100 $\mu m;$ C. Palp, scale bar = 100 $\mu m;$ D. Trochanter of palp, scale bar = 50 $\mu m;$ E. Tarsus I; F. Tarsus II. G. Tarsus III; H. Tarsus IV; scale bar on all tarsi = 100 $\mu m.$

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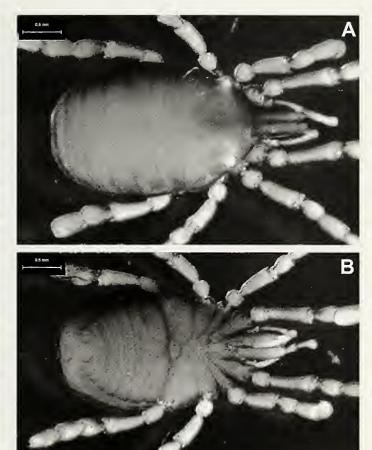




Figure 14.—*Austropurcellia vicina* n. sp., male holotype: A. Lateral view; B. Ventral view; C. Dorsal view. Scale bars = 500 μm.

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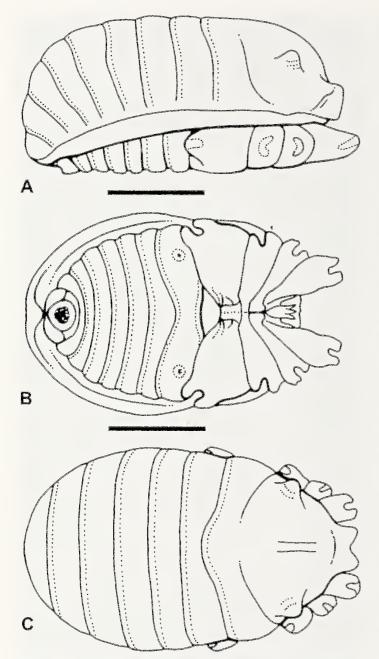


Figure 15.—Austropurcellia vicina n. sp., male holotype: A. Lateral view; B. Ventral view; C. Dorsal view. Scale bars = 500 µm.

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Appendix 1.—Catalogue of Austropurcellia specimens, excluding species described in the current study.

Austropurcellia arcticosa (Cantrell 1980)

Rakaia arcticosa Cantrell 1980:241.

Type locality.—AUSTRALIA: Queensland: Cooper Creek, ca 21 km N of Daintree River, 16°10'S,145°25'E (estimated), 14 November 1969, B.K. Cantrell (QM S334).

Paratype locality.—AUSTRALIA: *Queensland*: Noah Creek, 16°07'S,145°25'E, 21 June 1971, Taylor and Feehan, ANIC Berlesate No. 321 (ANIC).

Additional collection.—AUSTRALIA: *Queensland*: Cooper Creek, Daintree National Park, Cape Tribulation, 16°09′58″S,145°24′56.2″E, 15 February 2003, G. Giribet and C. D'Haese (MCZ DNA100951).

Austropurcellia capricornia (Todd Davies 1977) Neopurcellia capricornia Todd Davies 1977:61.

Type locality.—AUSTRALIA: Queensland: Finch Hatton, 21°8'S,148°37'E, (estimated), 10 April 1975, V. Davies and R. Kohout (QM W5765).

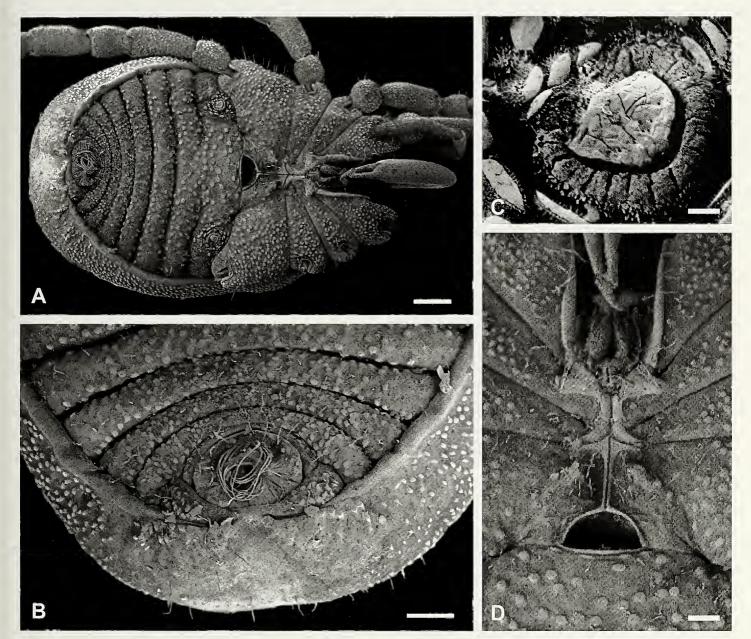


Figure 16.—Austropurcellia viciua n. sp., male paratype: A. Ventral view, scale bar = $200 \, \mu m$; B. Posterior ventral area, scale bar = $100 \, \mu m$; C. Spiracle, scale bar = $10 \, \mu m$; D. Gonostome and sternal area, scale bar = $100 \, \mu m$.

Austropurcellia daviesae (Juberthie 1989) Rakaia daviesae Juberthie 1989:499.

Type locality.—AUSTRALIA: *Queensland*: Graham Range, 17°17′S,145°57′E, 9 April 1979, G.B. Monteith, QM Berlesate No. 3 (QM S6441, S6442, S6443).

Additional collections.—AUSTRALIA: *Queeuslaud*: Bellenden Ker National Park, 40 km SSE. of Cairns, 17°15′S,145°54′E, 100 m, 20–21 January 1992, D. Burckhardt (#18a) (MHNG); 4 km E. of Lake Barine, 17°16′S,145°41′E, 700 m, 1 July 1971, Taylor and Feehan, ANIC berlesate 352 (ANIC); Lake Barrine, Crater Lakes National Park, Yungaburra, 17°14′44.2″S,145°38′31.5″E, 19 February 2003, G. Giribet and C. D'Haese (MCZ DNA 100947); Lake Eacham National Park, 17°17′S,145°37E (estimated), 25 May 1980, I.D. Naumann and J.C. Cardale, ANIC berlesate 681 (ANIC), mounted for SEM: Macalester stubs 2.3, 2.4; Rose Gums Wilderness Retreat; 17°18′51″S,145°42′08″E, 15 March 2006, G. Hormiga and L. Lopardo (MCZ DNA101953).

Austropurcellia forsteri (Juberthie 2000) Neopurcellia forsteri Juberthie 2000:149.

Type locality.—Australia: *Queeuslaud*: 3 km W of Cape Tribulation (site no. 6), 16°04′S, 145°25′E (estimated), January 1983, G.B. Monteith, berlese no. 512 (location of type specimens unknown).

Additional collection.—Australia: *Queeuslaud*: Emmagen Creek, Daintree National Park, 16°3′41.4″S,145°27′43.8″E, 18 February 2003, G. Giribet and C. D'Haese (MCZ DNA100945).

Austropurcellia scoparia Juberthie 1988 Austropurcellia scoparia Juberthie 1988:133.

Type locality.—AUSTRALIA: *Queenslaud*: 2 km N of Mount Lewis via Julatten, 1000 m, 16°34′S,145°17′E (estimated), 9 June 1981, G. Monteith and D. Cook, berlese No. 281 (MNHN).

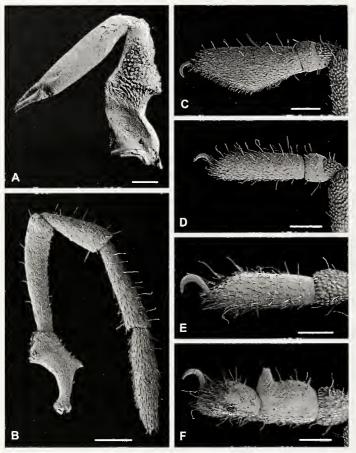


Figure 17.—*Austropurcellia vicina* n. sp., male paratype: A. Chelicera; B. Palp; C. Tarsus I; D. Tarsus II; E. Tarsus III; F. Tarsus IV. Scale bars = 100 μm.

Additional collections.—AUSTRALIA: *Queensland*: Mount Lewis Road, Julatten, 16°34′S,145°19′E (estimated),12 November 1975, A. Wallingford-Huggins (ANIC berlesate 508); Mount Lewis State Forest; 16°35′40.5″S, 145°16′45.5″E, 17 February 2003, G. Giribet and C. D'Haese (MCZ DNA100946).

Austropurcellia woodwardi (Forster 1955) Rakaia woodwardi Forster 1955:355.

Type locality.—AUSTRALIA: *Queensland:* Clump Point, Great Dividing Range, ex leaf mold, 17°51′S, 146°7′E (estimated), 3 June 1953, T.E. Woodward (QM).

Additional collection.—AUSTRALIA: *Queensland*: Tully Falls, 17°43'S, 145°32'E (estimated), 21 August 1953, W.A. McDougall (QM).

Collections of specimens that cannot be assigned to species (juveniles and females only).—AUSTRALIA: Queensland: Eacham National Park; 17°08'S, 145°37'E, 1-7 October 1972, R.W. Taylor (ANIC berlesates 428, 429); Eacham National Park, 760 m, 17°18'S, 145°47′E, 16 February 1973, R.W. Taylor (ANIC berlesate 436); Gap Creek, 5 km SSE of Mt. Finnegan, 15°50'S,145°20'E, 13-16 May 1971, A. Calder and J. Feehan (ANIC berlesate 736); Lake Barrine, transect on lower slope, Atherton Tablelands, Tullgrens site code F3#2, 17°15'S, 145°38'E (estimated), October–November 2000, H.C. Proctor (MCZ 98676); Malanda Scrub, Atherton Tablelands, Tullgrens site code F2, 17°19'S,145°30'E (estimated), October-November 2000, H.C. Proctor (MCZ 98674); Mount Edith, N of Tinaroo Falls Dam, 17°06'S,145a37'E (estimated), 19 May 1980, I.D. Naumann and J.C. Cardale (ANIC berlesate 677); near Mount Haig, 1140 m, 17°06'S, 145°35'E, 30 June 1971, Taylor and Feehan (ANIC berlesate 349); Mount Tiptree, 17°03'S,145°38'E, 29 June 1971, Taylor and Feehan (ANIC berlesate 345); Tucker (private land) above Johnstone River Gorge, Atherton Tablelands, Tullgrens site code F10, 17°29′S,145°41′E (estimated), October-November 2000, H.C. Proctor (MCZ 98672).

Morphometry and geographical variation of *Bothriurus bonariensis* (Scorpiones: Bothriuridae)

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Abstract. Diversification of morphological characteristics among geographically separated populations is particularly important in understanding evolutionary processes and is considered the early stage of allopatric speciation. In the present study, we investigated geographic variation in scorpion populations of *Bothriurus bonariensis* (Koch 1842). Our principal goal was to compare different populations of this species with regard to its distribution, analyzing somatic and genitalic characters. In Argentina, specimens of *B. bonariensis* from Entre Ríos and Corrientes Provinces are dark brown, while specimens from La Pampa have reddish coloration. Scorpions of this species from Brazil and south of Uruguay are totally black. Also, we observed variability in body size, some morphological characteristics of adult specimens (e.g., prosoma length, hand height, hand width, and telson height), and genitalic traits (e.g., hemispermatophore lamina length, basal and distal lamina width, dorsal fold length). Results indicate the presence of evident geographic variation: populations from Argentina show similar morphological patterns that differ from populations from Uruguay. We discuss these data in the context of the current phylogeographical and evolutionary knowledge of this species.

Keywords: Coloration, genitalia, scorpion

Diversification of morphological characteristics among geographically separated populations is particularly important and is considered the starting point of allopatric speciation. For this reason, data on this diversification process are useful for understanding evolutionary processes (Coyne and Orr 2004). Differences among habitats can generate a divergent selection of groups in nature, resulting in reproductive isolation of the populations (Mayr 1942, 1963; Schluter 2000). Although geographical variation of genitalic traits can also be very important at morphological and behavioral levels, the analysis of this important aspect has received less attention (Eberhard 1985; Reinhardt 2010). The environment can influence species directly or indirectly with regard to genitalic variation; therefore, it is expected that these features can vary in shape or size in different populations or geographic locations. Although it is difficult to identify geographic factors that cause these variations and to separate these factors from genotypic effects, it is known that local and clinal variations in genitalic characters do exist (Lachaise et al. 1981; Hribar 1994; Tatsuta & Akimoto 1998; Kelly et al. 2000; Jennions & Kelly 2002; Tatsuta et al. 2001). Studies performed in different groups of insects and arachnids have shown that populations of a single species can begin differentiating both morphologically and genetically as a result of allopatric distribution, reaching reproductive isolation afterwards (Yamashita & Polis 1995; Postiglioni & Costa 2006; Holwell 2008).

The present study approaches this subject in populations of the type species of the scorpion family Bothriuridae: *Bothriurus bonariensis* (Koch 1842). This species has a broad distribution in South America. It inhabits central Argentina, Uruguay, and southeastern Brazil (Mattoni & Acosta 2005; Ojanguren Affilastro 2005) (Fig. 1). In Argentina, this scorpion can be found in the Provinces of Buenos Aires, La Pampa, Córdoba, San Luis, Entre Ríos, Corrientes and possibly Santa Fe. This distribution corresponds to the Pampean

and Espinal phytogeographical provinces (Cabrera & Willink 1980; Ojanguren Affilastro 2005).

Within its range, some variations occur in the coloration patterns of specimens from different zones. In Argentina, specimens from the Provinces of Entre Ríos and Corrientes are dark brown, while specimens from La Pampa province have reddish coloration, with large areas lacking dark or blackish pigment. In contrast, specimens from Brazil and Uruguay are totally black (Mattoni 2003; Ojanguren Affilastro 2005). This notable variability in pigmentation was mentioned by San Martín (1962) for different populations in Uruguay. Similarly, great variability in body size of adult specimens has been observed. Individuals from the Espinal area of Córdoba are remarkably smaller than those from Uruguay and Brazil (Mattoni 2003). As in other animal groups (Alatalo et al. 1988; Moller 1991; Andersson 1994; Pomiankowski & Moller 1995), in this species there is great phenotypic variation in characters under sexual selection. For example, some structures of the spermatophore such as the lamina crest and the lateral fold show high coefficients of variation (Peretti et al. 2001). In addition, spermatophores and hemispermatophores of male B. bonariensis from Córdoba (at the margin of the range of the species) show higher fluctuating asymmetry than specimens from the southern part of Buenos Aires Province (Peretti et al. 2001; Peretti unpublished data). Nevertheless, these differences have not been compared with populations from other areas.

The main objective of this work is to compare different populations of *B. bonariensis* within its range (from Córdoba, Entre Ríos, and Buenos Aires Provinces in Argentina and north and south of Uruguay) by analyzing somatic and genitalic characters. We expected to find morphometric variation among populations that was related to the general geographical distribution of the species.

METHODS

Study Material.—We used specimens from the following collections: Museo Argentino de Ciencias Naturales, Buenos

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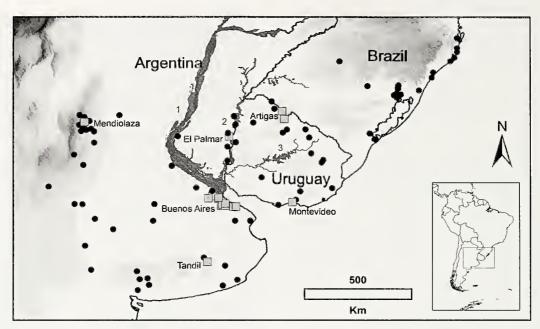


Figure 1.—Geographic distribution of *Bothriurus bonariensis*. Black circles indicate the total distribution of the species known to date; gray squares show the populations used in this work. Major water bodies are displayed in gray (1: Paraná River, 2: Uruguay River, 3: Negro River).

Aires (MACN); Laboratorio de Biología Reproductiva y Evolución, Córdoba (LBRE); and A. V. Peretti's personal collection (AVP). We analyzed specimens of B. bonariensis that were selected from different distant populations (Fig. 1). We only selected populations from which materials were abundant (allowing statistical comparisons) and well preserved (poor preservation techniques alter the pigmentation pattern and the hemispermatophore form). These populations were examined: Mendiolaza (Córdoba Province, Argentina - 31°16′0.01″S, 64°18'0.04"W, 543 m.a.s.l.): 20 males, 11 females, 20 pairs of hemispermatophores; Tandil (Buenos Aires, Argentina -37°19′4.12″S, 59°09′1.41″W, 190 m.a.s.l.): 7 males, 3 females, 6 pairs of hemispermatophores; La Plata and surroundings of Buenos Aires (Buenos Aires Province, Argentina -34°36′30.3″S, 58°22′23.38″W, 26 m.a.s.l.): 10 males, 5 females, 8 pairs of hemispermatophores; El Palmar National Park (Entre Ríos Province, Argentina - 31°51'11"S, 58°19'21"W, 21 m.a.s.l.): 8 males, 9 females, 8 pairs of hemispermatophores; Artigas and surroundings (Artigas Province, Uruguay 30°29′17.12″S, 57°6′4.75″W, 85 m.a.s.l.): 9 males, 3 females, 7 pairs of hemispermatophores; Piedras de Afilar (Canelones Province, Uruguay – 34°44′S, 55°35′W, 57 m.a.s.l.): 5 males, 4 females, 5 pairs of hemispermatophores (Fig. 1) (complete list of materials in Appendix I).

Characterization of the population.—Preparation of the sample and criteria for morphological analysis: To compare different populations of B. bonariensis, we followed the biological species concept (Mayr 1942). All specimens studied were preserved in 80% ethanol. Hemispermatophores were extracted, cleaned and preserved following Sissom et al. (1990). Color characteristics and patterns of pigmentation between populations were compared based on San Martín (1962) and Ojanguren Affilastro (2005). Measurements of quantitative characters of external morphology were taken on the right side in both males and females and on the left hemispermatophore in males (Peretti et al. 2001). The

measurements were taken with a Nikon SMZ1500 stereomicroscope with ocular micrometer, equipped with a Nikon Sight DS-Fil digital camera. Digital pictures were analyzed with the Image Tool 3.0 measuring software (© UTHSCSA 1996–2002). We examined 28 morphometric characters (20 in males, 8 in females) (Table 1, Fig. 2).

Morphometrics: We performed all the statistical analyses with the PC program NCSS 2007 (© Hintze 2007). Results of measurements of each character were analyzed with analysis of variance tests (ANOVA or Kruskal-Wallis one-way ANOVA, depending of the normality of the data) and a posteriori tests (Tukey-Kramer or Dunn). Although it is unknown whether post-reproductive molting after reaching the adult stage occurs in scorpions (Polis & Sissom 1990), the adults of

Table 1.—Body and hemispermatophore characters measured in the *Bothriurus bonariensis* populations. The numbers of each character correspond to those shown in Figure 2.

Body Characters	Length	Width	Height	Calculated proportions
Prosoma	1			1/2
Hand	2	3	4	1/4
Movable finger	5			2/4
Telson	6		7	2/5
Pecten	8			6/7
Hemispermatophores				
Lamina	9	10 (distal)		1/9+13
		11 (median)		
		12 (basal)		
Trunk	13			9/11
Capsular lobe	14	15		9/13
Dorsal fold	16	17		14/9+13
Dorsal fold-Lateral edge space	18			18/9
Lateral edge	19			20/9
Crest	20			

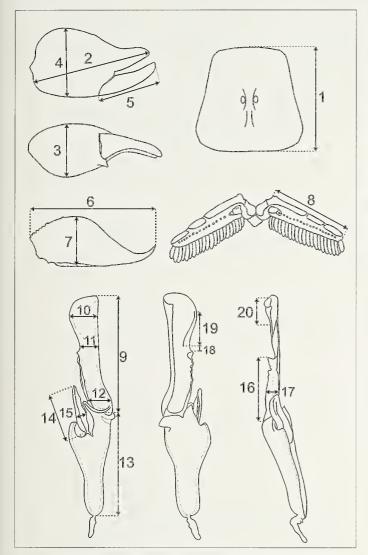


Figure 2.—Body and genitalic characters measured in the samples. The numbers refer to character number cited in Table 1.

B. bonariensis show a wide range of sizes (Peretti et al. 2001), as observed in other scorpions and spiders (Savory 1977; Polis & Sissom 1990; Benton 1992). For this reason, besides comparing the mean values of absolute measurements for each character, we calculated indices that show proportions of some of the body traits compared to others. These proportion values are shown in Table 1.

Multivariate analyses: To compare populations, including all the selected variables, we performed a multivariate discriminant analysis (Fisher 1936). Discriminant analysis results in a set of prediction equations (linear functions) based on independent variables that are used to classify individuals into groups. As an estimating method, the discriminant linear function was used without setting a priori probabilities and without selecting variables. Canonical Correlation Coefficient and Wilks' Lambda were calculated, which allowed us to choose the most appropriate model among all models that can be constructed from the variables previously selected. Both ratios measure the differences between the groups due to discriminant functions. This technique allowed us to analyze whether there was

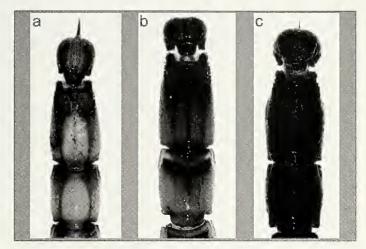


Figure 3.—Pigmentation patterns found on the ventral side of the metasoma in different populations of *Bothriurus bonariensis*. a) two darker lateral lines in all segments; b) two darker lines, one central line in the fifth segment and/or central patches in all segments; c) dark uniform coloration in all segments.

a separation among populations, considering the variables studied, and to determine the influence of each variable on the population discrimination level (Brown & Wicker 2000; Vignoli et al. 2005).

RESULTS

External morphological analysis.—We observed remarkable variation in the color of the specimens from different populations. Coloration of individuals in the Mendiolaza population was brownish-gray, the same as in individuals from the Buenos Aires and Tandil populations. Specimens from the El Palmar population showed dark and reddish coloration, which was darker in the specimens from Artigas population. Piedras de Afilar specimens were totally black. There were three different pigmentation patterns in the metasoma ventral area: A) two dark lateral lines in all segments of the metasoma; B) two dark lines, one central line in the fifth segment and/or central patches in all the segments; or C) dark uniform coloration in all the segments (Fig. 3).

Scorpions from the Mendiolaza and Tandil populations manifested Pattern A in all cases. Individuals from Piedras de Afilar showed only Pattern C. Specimens from Buenos Aires and El Palmar displayed different percentages of Patterns A and B (Buenos Aires: 60% A, 40% B; El Palmar: 50% A, 50% B), whereas in the population from Artigas, all three patterns occurred (40% A, 25% B, 35% C).

Morphometric analysis.—Varying body measurements reflected separation of the populations studied in three groups: specimens from Mendiolaza were smaller, specimens from Piedras de Afilar were larger, and specimens from other populations showed intermediate sizes (Tables 2, 3).

Body index also indicated significant division among populations in almost all the variables (Table 4). The relative height of the hand in relation to hand length (hand height/hand length) in males and females was greater in individuals from Piedras de Afilar (Figs. 4a, b). Similarly, length and height of the hands were greater in relation to prosoma length in Piedras de Afilar compared to other populations. These variables

Table 2.—Mean values (mm) and standard deviations of the male's body and hemispermatophore traits measured in six populations of Bothriums bonariensis. Separation according

ž	Body characters	Mendiolaza	Tandil	La Plata and Buenos Aires suburbs	El Palmar National Park	Artigas and surrounds	Piedras de Afilar	df.	Statistics	Ь
_	Prosoma length	5.26 ± 0.10	5.59 ± 0.17	5.52 ± 0.14	5.75 ± 0.16	5.53 ± 0.15	6.08 ± 0.20	5, 58	F = 3.34	0.01
2	Hand length	7.22 ± 0.12	7.71 ± 0.20	7.69 ± 0.17	7.55 ± 0.19	7.52 ± 0.18	7.78 ± 0.24		H = 9.13	0.1
33	Hand width	2.38 ± 0.06	2.87 ± 0.10	2.85 ± 0.08	2.66 ± 0.10	2.69 ± 0.09		5, 58	Ш	< 0.001
		A	В	В	AB	AB	В			
4	Hand height	3.50 ± 0.08	4.20 ± 0.14	4.08 ± 0.12	4.09 ± 0.13	4.09 ± 0.12	4.60 ± 0.17	5, 58	H = 29.85	<0.001
8	Movable finger length	$\frac{A}{3.76 \pm 0.06}$	B 4.16 ± 0.10	$\frac{1}{8}$ 8.15 ± 0.08	$\frac{1}{6}$ 8.15 ± 0.10	$\frac{1}{8}$ 8.15 ± 0.09	$\frac{1}{8}$ 8.02 ± 0.12	5. 58	H = 20.53	<0.001
	0	A	AB	В	В	В	AB			
9	Telson length	6.04 ± 0.10	6.50 ± 0.17	6.53 ± 0.15	6.43 ± 0.16	6.23 ± 0.15	7.24 ± 0.21	5, 58	H = 20.35	0.001
7	Telson height	A 1 84 + 0 04	AB 2 22 + 0 07	AB 2 20 + 0 06	AB 2 12 + 0 07	AB 2.05 + 0.07	B 2 83 + 0 09	5 58	H = 33.16	<0.00
		\ \ \	<u> </u>	B ::-	AB	AB) A			2000
∞	Pecten length Genitalic characters	4.66 ± 0.15	4.74 ± 0.26	4.71 ± 0.24	5.01 ± 0.24	4.96 ± 0.23	4.94 ± 0.31	5, 56	H = 12.33	0.03
6	Lamina length	5.11 ± 0.06	5.78 ± 0.10	5.53 ± 0.08	5.55 ± 0.10	6.11 ± 0.09	6.16 ± 0.11	5, 50	F = 26.20	<0.001
		Ą	BC	O	ပ	В	В			
10	Lamina width (distal)	1.51 ± 0.03	1.65 ± 0.05	1.57 ± 0.04	1.61 ± 0.05	1.66 ± 0.09	1.69 ± 0.11	5, 50	F = 3.58	0.008
-		A 000	AB	AB	AB	AB	10 C C C			t c
1 2	Lamina width (median)	0.99 ± 0.02	1.04 ± 0.04	1.01 ± 0.03	0.99 ± 0.04	1.08 ± 0.04	1.10 ± 0.04	, 5, 5 <u>1</u>	F = 1.62	0.17
1	Laiiiiia wiuii (Uasai)	1.20 ± 0.02 A	1.23 ± 0.03 AB	1.23 ± 0.03 AC	AB	1.30 ± 0.03 B	1.37 ± 0.04 BC		I	/0.001
13	Trunk length	4.31 ± 0.07	4.29 ± 0.18	4.53 ± 0.13	4.54 ± 0.13	4.42 ± 0.12	5.09 ± 0.14	5, 46	H = 13.97	0.01
		Ą	AB	AB	AB	AB	В			
4	Capsular lobe length	2.46 ± 0.05 A	2.34 ± 0.08 B	2.42 ± 0.07 B	2.36 ± 0.08 B	3.00 ± 0.08 B	2.50 ± 0.09 B	5, 51	H = 13.13	0.05
15	Capsular lobe width	0.49 ± 0.01	0.48 ± 0.02	0.49 ± 0.02	0.47 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	5, 51	H = 15.95	0.006
		AB	AB	AB	Α	B	AB			
16	Dorsal fold length	2.49 ± 0.04 A	2.99 ± 0.08 B	2.78 ± 0.07 AB	2.78 ± 0.08 AB	3.50 ± 0.08 B	3.19 ± 0.09 B	5, 51	H = 40.06	<0.001
17	Dorsal fold width	0.60 ± 0.01	0.61 ± 0.02	0.64 ± 0.02	0.62 ± 0.02	0.66 ± 0.02	0.69 ± 0.03	5, 51	H = 11.24	0.04
		¥	AB	AB	AB	AB	æ			
∞_	Distance of dorsal fold—	0.47 ± 0.04	0.29 ± 0.07	0.38 ± 0.06	0.22 ± 0.07	0.16 ± 0.06	0.42 ± 0.07	5, 50	F = 4.87	0.001
19	Lateral edge length	1.23 ± 0.05	1.60 ± 0.09	1.37 ± 0.07	1.60 ± 0.09	1.60 ± 0.08	1.40 ± 0.09	5.50	H = 20.74	<0.001
	,	A	В	AB	М	В	AB			
20	Crest length	0.95 ± 0.05	1.08 ± 0.08	1.03 ± 0.07	1.06 ± 0.08	1.07 ± 0.08	1.06 ± 0.09	5, 49	H = 10.78	90.0

Table 3.—Comparison of females of different populations of Bothriums bonariensis in terms of average values (mm) and standard deviations of the variables used. Separation

acco	according to ANOVA test (F statistic) and Kruskal-Wallis (H	tistic) and Kruska	ıl-Wallis (H statis	statistic). The grouping and separation of populations by Tukey-Kramer and Dunn tests is indicated by A, B, and C.	paration of popul	ations by Tukey	-Kramer and Du	unn tests is	indicated by A	B, and C.
ž	Body characters	Mendiolaza	Tandil	La Plata and Buenos Aires suburbs	El Palmar National Park	Artigas and surrounds	Piedras de Afilar	df	Statistical	Р
	Prosoma length	5.2 ±0.18	5.50 ±0.35	5.81 ±0.27	5.19 ± 0.20	6.26 ± 0.35	5.51 ±0.30	5, 34	F = 2.12	0.09
7	Hand length	6.87 ± 0.20 B	7.00 ± 0.38 AB	7.47 ± 0.33 AB	6.76 ± 0.21 B	8.26 ± 0.38 A	6.60 ± 0.33 B	5, 33	F = 3.27	0.01
m	Hand width	1.98 ± 0.07 B	2.21 ± 0.14 AB	2.37 ± 0.11 AB	2.12 ± 0.08 B	2.73 ± 0.14 A	2.18 ± 0.12 AB	5, 34	F = 5.44	0.001
4	Hand height	2.72 ± 0.10 B	2.94 ± 0.20 AB	3.18 ± 0.16 AB	2.99 ± 0.12 B	3.77 ± 0.20 A	3.00 ± 0.17 AB	5, 34	F = 4.54	0.003
S	Movable finger length	3.69 ± 0.11 B	3.99 ± 0.21 AB	4.03 ± 0.16 AB	3.68 ± 0.12 B	4.68 ± 0.21 A	3.48 ± 0.18 B	5, 34	F = 4.96	0.002
9	Telson length	5.32 ± 0.17 B	5.43 ± 0.32 AB	5.43 ± 0.19 AB	6.04 ± 0.25 B	6.76 ± 0.32 A	5.61 ± 0.28 AB	5, 34	F = 3.93	0.007
7	Telson height	1.67 ± 0.07 A	1.97 ± 0.14 ABC	2.11 ± 0.11 BC	1.88 ± 0.08 AC	2.44 ± 0.14 B	1.98 ± 0.12 ABC	5, 34	F = 5.45	0.001
∞	Pecten length	3.65 ± 0.14	3.89 ± 0.28	4.03 ± 0.24	3.87 ± 0.17	4.64 ± 0.28	3.90 ± 0.24	5, 32	F = 2.03	0.11

Table 4.—Body indices calculated for male and female populations of *Bothriurus bonariensis*. Separation according to ANOVA test (F statistic) and Kruskal-Wallis (H statistic).

Indices of proportions	df	Statistical	P
Males			
Prosoma length/Hand length	5, 58	H = 11.49	0.04
Hand length / Hand height	5, 58	F = 22.13	< 0.001
Hand length /Movable finger length	5, 58	F = 4.65	0.001
Prosoma length / Hand height	5, 58	H = 30.65	< 0.001
Telson length/ Telson height	5, 58	F = 12.41	< 0.001
Prosoma length /Hemispermatophore	5, 45	H = 12.01	0.03
length			
Lamina length/ Lamina width (median)	5, 50	F = 4.64	0.002
Lamina length /Trunk length	5, 45	H = 19.48	0.002
Capsular lobe length/	5, 45	H = 11.77	0.04
Hemispermatophore length			
Dorsal fold length/ Lamina length	5, 50	F = 13.41	< 0.001
Crest length/ Lamina length	5, 49	H = 2.02	0.8
Females			
Prosoma length / Hand length	5, 33	H = 11.55	0.04
Hand length / Hand height	5, 33	H = 23.93	
Hand length / Movable finger length	5, 33	F = 3.54	0.01
Prosoma length / Hand height	5, 34	F = 2.55	0.05
Telson length / Telson height	5, 34	H = 11.87	

signify that the hands were larger and broader, giving a more robust aspect to the specimens from the Piedras de Afilar population. In the Mendiolaza population, these structures were slimmer. The telson was also narrower related to its length (telson width/length) in Mendiolaza and more spherical in specimens from Piedras de Afilar (Figs. 4c, d). The other populations showed overlap among them, but were different in comparison with the Mendiolaza and Piedras de Afilar populations.

In male genitalia, indices in relation to prosoma length showed that the hemispermatophores in the Piedras de Afilar population were longer compared to the other populations (Fig. 5a). The lamina was shorter and broader in the Mendiolaza population and longer and slimmer in the individuals from the other populations (Fig. 5c). The capsular lobe was also longer compared to the total length of the hemispermatophore in the Piedras de Afilar population and shorter in the Mendiolaza population. The same pattern occurred with the dorsal fold of the lamina in relation to the lamina length in the Artigas population (Figs. 5b, d).

Multivariate analysis.—The discriminant multivariate analysis showed that, considering all the variables, there was a clear discrimination among the populations. In males, we found a significant separation between the Mendiolaza and Uruguay populations. These populations differed from the rest and between themselves as well. The Buenos Aires, Tandil, and El Palmar populations comprised a single group. The major discrimination occurred in the space of 1 and 2 scores for which the Wilk's Lambda values were 0.00085 and 0.016, respectively (Canonical discriminant analysis; F: 3.3 and 2.1, P < 0.001; Fig. 6a). For the rest of the scores, there was no separation.

As shown in Table 5, the variables that most influenced separation among males were two genitalic traits, the dorsal fold length and the lamina length of the hemispermatophore,

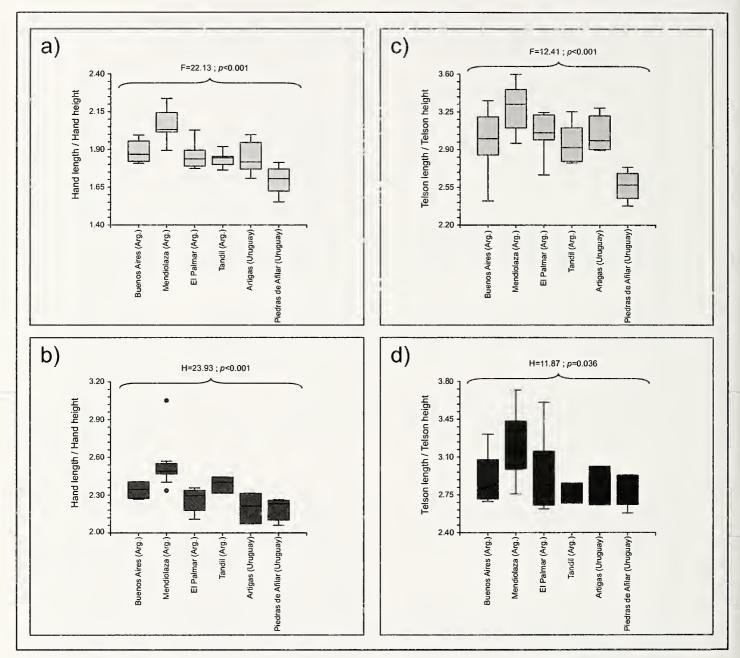


Figure 4.—Separation by means of box plots in *Bothriurus bonariensis* populations: a) hand length vs. hand height in males (ANOVA, $F_{5,58}$ = 22.13, P < 0.001); b) hand length vs. hand height in females (Kruskal-Wallis One-Way ANOVA, $H_{5,33}$ = 23.93, P < 0.001); c) telson length vs. telson height in males (ANOVA, $F_{5,58}$ = 12.41, P < 0.001); d) telson length vs. telson height in females (Kruskal-Wallis One-Way ANOVA, $H_{5,34}$ = 11.87, P = 0.036).

followed by body variables such as telson height and hand height and width.

In females, the differences were not so striking. With regard to males, the Mendiolaza population comprised one group, the Piedras de Afilar population another group, and the remaining populations a third group.

Scores that better explained the variation were 1 and 2 with Wilk's Lambda values of 0.018 and 0.08, respectively (Canonical discriminant analysis; F: 3.2 and 2.6, P < 0.001; Fig. 6b). Female variables that most influenced population differentiation were telson height and characters such as hand width and hand height, and length of the movable finger (Table 5).

DISCUSSION

Results indicate that intraspecific geographic variation occurs in *B. bonariensis*, both in morphological and genitalic characters. Populations from Argentina share morphological features that are notably different from features of the Uruguayan populations. In Argentina, the specimens from the Mendiolaza population are smaller and present slimmer and more delicate structures. Individuals from this population also have shorter hemispermatophores as well as shorter capsular lobes and dorsal folds, but broader lamina. The other populations in Argentina (Tandil, Buenos Aires, and El Palmar) do not differ among themselves, but comprise a different group (larger body size and slightly darker coloration).

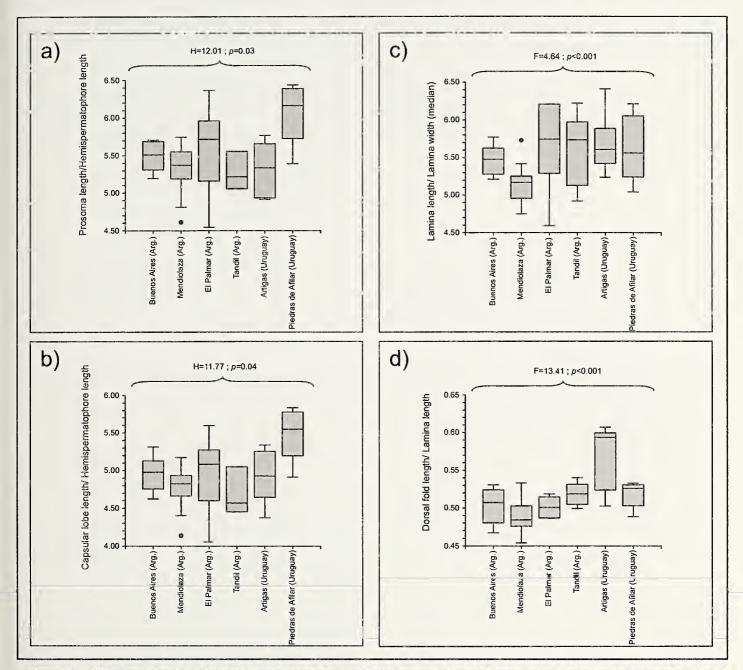


Figure 5.—Differences in male genitalia in the six populations of *Bothriurus bonariensis*: a) prosoma length vs. hemispermatophore length (Kruskal-Wallis One-Way ANOVA, $H_{5,45} = 12.01$, P = 0.03); b) capsular lobe length vs. hemispermatophore length (Kruskal-Wallis One-Way ANOVA, $H_{5,45} = 11.77$, P = 0.04); c) lamina length vs. lamina width (ANOVA, $F_{5,50} = 4.64$, P < 0.001); d) dorsal fold length vs. lamina length (ANOVA, $F_{5,50} = 13.41$, P < 0.001).

Scorpions from the Uruguay populations are more robust. The hemispermatophores in the Piedras de Afilar population are larger and possess slimmer and longer laminas. Specimens from northern Uruguay (Artigas) differ in size and, in agreement with San Martin (1962), their color is different from that in the southern Uruguay population (i.e., Piedras de Afilar). Indeed, The Uruguayan populations are larger with more spherical structures and completely black coloration (specimens from Artigas are reddish). Their hemispermatophores have shorter lamina and longer capsular lobes.

According to the discriminant analysis, genitalic variables in males allow for better differentiation among populations. This

is very important from taxonomic and evolutionary points of view, because when high sexual selection pressures occur, genitalic traits tend to show rapid divergence compared to other morphological traits (Eberhard 1985, 2010; Hosken & Stockley 2003).

Regarding pigmentation variation, in the Mendiolaza and Tandil populations all specimens show the same pattern of two darker lateral lines in the ventral zone of the metasoma. In addition, the Buenos Aires and El Palmar populations display another pattern of dark lateral lines and many central lines. In contrast, the Piedras de Afilar specimens manifest a uniform dark coloration. Specimens from the Artigas population show

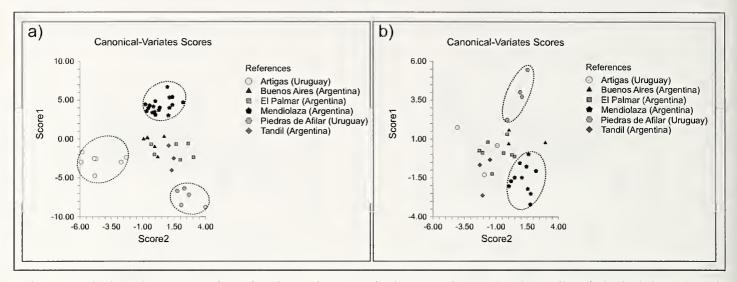


Figure 6.—Discrimination of populations of *Bothriurus bonariensis* in the space of scores 1 and 2 as Canonical Discriminant Analysis: a) males: Score 1: F: 3.3, P < 0.001, Wilk's lambda: 0.00085; Score 2: F = 2.1, P < 0.001, Wilk's lambda: 0.016; b) females: Score 1: F: 3.2, P < 0.001, Wilk's lambda: 0.018; Score 2: F = 2.6, P < 0.001, Wilk's lambda: 0.08.

all three patterns of pigmentation. Thus, not only the general coloration but also pigmentation patterns turn gradually more pigmented from Mendiolaza to Uruguay. This variation could be related to environmental characteristics that change throughout the species' range. The climate gets wetter and precipitation levels increase from west to east (data from Servicio Metereologico Nacional, online at www.smn.gov.ar, and Direccion Nacional de Metereologia de Uruguay, online at

Table 5.—Influence of variables considered in the Canonical Discriminant Analysis for group separation in males and females of the studied populations of *Bothriurus bonariensis*.

Influence of variables	in males	
Variable	F	P
Males		
Dorsal fold length	29.39	< 0.001
Lamina length	28.71	< 0.001
Telson height	22.81	< 0.001
Hand height	11.25	< 0.001
Hand width	9.22	< 0.001
Capsular lobe length	8.95	< 0.001
Movable finger length	6.52	< 0.001
Telson length	6.26	< 0.001
Lamina width (basal)	5.65	< 0.001
Lateral cdge length	5.46	< 0.001
Trunk length	5.15	0.001
Distance of dorsal fold-lateral edge	4.05	0.004
Prosoma length	3.80	0.007
Lamina width (distal)	3.24	0.015
Capsular lobe width	2.90	0.025
Dorsal fold width	2.49	0.047
Females		
Telson height	5.45	0.001
Hand width	5.00	0.002
Movable finger length	4.45	0.005
Hand height	4.26	0.006
Telson length	3.49	0.015
Hand length	3.09	0.026

www.metereologia.com.uy). It is known that scorpion species belonging to wet zones show a greater amount of pigment than species inhabiting drier zones (Lourenço & Cloudsley-Thompson 1996; Mattoni 2002), and in this case, the patterns found in the present study could be caused for the same reason.

In consideration of all these patterns, variability in morphology of specimens from different populations related to the total distribution of the species could result in a gradient of size, coloration, morphological features (prosoma length, hand height and width, movable finger length and telson height), and genitalic traits (lamina length, basal and distal lamina width, dorsal fold length). This gradient may indicate that scorpions become larger and acquire more robust structures and darker coloration in populations located more eastward.

However, it is not possible to confirm the existence of an environmental cline or morphological gradation along the distribution of the species until new and abundant materials from intermediate populations become available. The samples from intermediate locations between the populations studied in Argentina are scarce, and most of them are extremely old and poorly preserved (preservation alters the pigmentation pattern and the hemispermatophore form). In fact, most of the intermediate localities that the species inhabit are now strongly anthropized, this region being the main agriculture area in Argentina (the "pampas," with more than 60% of the land planted with permanent crops; see www.fao.org for map). Almost all of the natural habitats in the humid "pampas" and "espinal" region have disappeared (Dinerstein et al. 1995), and finding abundant populations of fossorial scorpions, such as B. bonariensis, is difficult.

It is known that scorpions have limitations in moving great distances and adapting to particular types of environments (soils, climatic conditions, and vegetation: Polis 1990; Prendini 2001). Therefore, historical biogeographic processes could have led to population differentiation in *B. bonariensis*. During the Miocene era, successive marine transgressions of the

Atlantic and Pacific Oceans over South America made a maritime channel that separated terrestrial environments. This channel extended over the greater part of Argentina, western Uruguay, southern Paraguay, and southeastern Bolivia (Donato et al. 2003; Donato 2006). This could have resulted in a vicariant process of populations of *B. bonariensis*, possibly isolating terrestrial environments that these organisms inhabited. Additionally, current biogeographical barriers such as the Uruguay River between Argentina and Uruguay, and the Negro River within Uruguay (Fig. 1), could be important causes of divergence among the groups analyzed (Postiglioni & Costa 2006).

Some complete matings between mixed couples from the Buenos Aires and Mendiolaza populations and between the Piedras de Afilar and Mendiolaza populations have been observed (Peretti 1993; Olivero unpub. data). However, some problems during the last stages of mating have been detected (Olivero unpub. data). These problems could be due to some of the morphometric differences in the genitalia between specimens already discussed. Together with the current barriers, this incipient "mechanical incompatibility" could result in a reduction of gene flow between the most distant populations, and each of them could be gathering unique characteristics and becoming differentiated entities. This could be generating speciation, and each group could evolve in an independent way resulting in the differentiation of a new species or subspecies. Nevertheless, it is not possible to confirm this yet, as the genetic structure in each of the species studied is unknown. This generates new questions and shows the need for further studies and genetic analysis, with additional observations of mating behavior between populations, to enrich the present knowledge from phylogeographical and evolutionary perspectives.

ACKNOWLEDGMENTS

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APPENDIX 1

Material of Bothriurus Bonariensis Examined.

ARGENTINA: Córdoba, Departamento Colón, Mendiolaza, 1995. A.A. Peretti & S. Castelvetri, 13 (AVP); 27 January 1996, A.A. Peretti & S. Castelvetri, 13 (AVP); 27 December 1996, A.A. Peretti & S. Castelvetri, 73, 29 (AVP); 8 January 1997, A.A. Peretti & S. Castelvetri, 23, 59 (AVP); 9 January 1997, A.A. Peretti & S. Castelvetri, 93, 49 (AVP). Buenos Aires, Tandil, 5 February 1945, M.L. Cambio, 13 (MACN); February 1947, Prosen, 23, 19 (MACN); 27-28 November 1967, E. Maury, 33 (MACN); 8 December 1969, M.E. Galiano, J. Poirot, 13 (MACN); January 1982, M.J. Ramírez, 29 (MACN). La Plata, 8 January 1973, C. Cesar, 1d (MACN); 18 February 1974, M. Milano, 18 (MACN). Gran Buenos Aires, Partido de Ezeiza, 9 January 1969, Gozzi, 1º (MACN); Tristán Suárez, 12 February 1997, D. Folgar, 13 (MACN); 3 January 1998, C. Velazquez, 19 (MACN); 11 January 1998, M. Aberbuj, 18 (MACN). Partido de Almirante Brown, Glew, 1974, J. Carpintero, 13 (MACN); Adrogué, 5 February 1998, J.M. Morel, 13 (MACN). Partido de Escobar Garín, 10 February 1997, E. Maidana, 1º (MACN). Partido de Luján, 5 km de Luján, 25 December 1983, I. Vazquez, 13 (MACN). Partido de San Miguel, Bella Vista, January 1995, T.M. Gallardo, 18 (MACN). Partido de Moreno, April 1998, Rapetti, 18 (MACN); Country San Diego, January 1996, M. Lavista, 18 (MACN); Country San Diego, 2 February 1998, M. Lavista, 1º (MACN). Partido de Pilar, 25 April 1997, D. Castella, 1º (MACN). Entre Ríos, Departamento Colón Parque Nacional El Palmar, Arroyo El Palmar, 20 January 1967, E. Maury, 13 (MACN); April 1974, A. Toth, 29 (MACN); February 1981, P. Goloboff, 19 (MACN); November 1982, collector unknown, 2º (MACN); October-November 2003, collector unknown, 13, 19 (MACN); Camino a Arroyo El Palmar, 14 December 2005, A. Ojanguren Affilastro, F. Labarque & C. Mattoni, 1♂, 1♀ (MACN); Camino a Arroyo El Palmar, 14 December 2005, A. Ojanguren Affilastro, F. Labarque & C. Mattoni, 43, 29 (MACN); Camping, under log, 16 December 2005, C. Mattoni, A. Ojanguren Affilastro & F. Labarque, 13 (LBRE). URUGUAY: Departamento Artigas, Arroyo de La Invernada, under stone, 18 February 1954, San Martín, 19 (MACN); under stone, 19 February 1954, San Martin, 13 (MACN). Departamento Rivera, Ruta 30, km 233, aprox. 100 km S de Artigas, under stone, 345 m, 13 December 2005, A. Ojanguren Affilastro, F. Labarque & C. Mattoni, 73, 19 (MACN); Ruta 30, km 233, aprox. 100 km S de Artigas, under stone, 13 December 2005, C. Mattoni, A. Ojanguren Affilastro & F. Labarque, 13, 12 (LBRE). Departamento Canelones, Piedras de Afilar: under stone, s/fecha, C. Toscano-Gadea, 13, 39 (LBRE); under stone, 12 December 2008, P. Olivero, D. Vrech & C. Toscano-Gadea, 43,1 9 (LBRE).

SHORT COMMUNICATION

Preliminary survey of the setal and sensory structures on the pedipalps of camel spiders (Arachnida: Solifugae)

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Abstract. Solifuges, or camel spiders (order Solifugae), keep their pedipalps extended when moving through the environment, utilizing them much the way insects use their antennae. The male also uses his pedipalps during copulation, staying in contact with the female throughout the process. The pedipalps are covered with setae that are assumed to function as chemo-, mechano-, thermo-, hygro-, and olfactory receptors. We surveyed setal forms and other possible sensory structures on the pedipalps of solifuges to determine 1) if certain setae and structures are common to all families, 2) if some may be unique to certain families, and 3) the possible function of the various setae and other structures. We found that all families had bifurcated and tapered setae, and that all families had dorsal tarsal pores. Other setal forms were evident only in one or a few families. Three of the setal types had distal pores suggesting that they function as chemoreceptors. These data suggest that the pattern and types of setae on the pedipalps of solifuges may be phylogenetically informative and confirm that the pedipalps do function as sensory appendages.

Keywords: Chemoreeeptor, mechanoreceptor, sensory receptor, Blumenthal organ, tarsal organ

Arachnologists have studied solifuges for decades, yet solifuge biology is still elusive (Punzo 1998). In particular, only a few studies have been carried out to elucidate the functional significance of morphological structures unique to these arachnids (Bertkau 1892; Roewer 1934; Junqua 1966; Brownell & Farley 1974; Haupt 1982; Bauchhenss 1983; Cushing et al. 2005; Klann et al. 2005, 2008; Klann & Alberti 2010).

The pedipalps, in particular, are in need of morphological study. Solifuges keep their pedipalps anteriorly extended when moving through the environment (Punzo 1998). They utilize them during hunting, as they have suctorial organs to help bring prey closer to their chelicerae (Cushing et al. 2005; Klann et al. 2008; Willemart et al. 2011). Males also use their pedipalps during mating, staying in contact with the females through the entire process, suggesting that there may be structures on the pedipalps functioning in intraspecific communication. Haupt (1982) looked at the morphology of chemotactile setae on the second and third legs of solifuges, and Bauchhenss (1983) examined the morphology and ultrastructure of sensilla ampullacea on the pedipalps. Beyond these studies and those on the suctorial organ (Cushing et al. 2005; Klann et al. 2008), little other work has been done on the sensory structures found on the appendages of solifuges. The

objective of this study was to carry out a preliminary survey of the setae and other possible sensory structures found on the pedipalps of 12 species representing each of the 12 families in the order.

We used Scanning Electron Microscopy (SEM) to examine the setal morphology of the pedipalps of solifuges that represent the 12 families of the order (Table 1). We used a FEI Quanta 450 Field Emission Gun at the U.S. Geological Service (USGS) Denver Microbeam Laboratory. To prepare each specimen, we cut off the right pedipalp at the coxus, washed off any obvious dirt with absolute ethanol and sonicated the pedipalp in absolute ethanol for 30-45 seconds. We then allowed the pedipalp to air dry before examining it under the light microscope to make sure visible impurities were minimal. Depending on the size of the pedipalp, we either mounted the pedipalp on a 12.5 mm diameter aluminum stub or on a glass slide. The pedipalps were mounted with double-sided sticky carbon tape. We used the USGS Microbeam Lab protocol to gold sputter the stubs for 35-45 seconds and then placed them into the SEM for examination. We photographed an entire view of each segment of the pedipalp in order to pinpoint setae of interest (Fig. 1A). We then magnified and photographed individual setae (Fig. 1B). Next, we focused on the tip and the base of each unique seta (Figs. 1C, 1D).

Table 1.—Specimens used for SEM analysis. AMNH = American Museum of Natural History, CAS = California Academy of Sciences, DMNS = Denver Museum of Nature and Science, SMN = National Museum of Namibia.

Stub #	Specimen #	Family	Species
Am1	DMNS ZA.23498	Ammotrechidae	Branchia angustus Muma 1951
Ce1	SMN 13632	Ceromidae	Ceroma inerme Purcell 1899
Dal	SMN 13278	Daesiidae	Biton browni (Lawrence 1965)
Er1	DMNS ZA.22647	Eremobatidae	Eremobates pallipes (Say 1823)
Ga1	AMNH 4624	Galeodidae	Galeodes olivieri Simon 1879
Gy1	SMN 13632	Gylippidae	Trichotoma michaelseni (Kraepelin 1914)
He1	AMNH 5768	Hexisopodidae	Chelypus barberi Purcell 1902
Ka1	AMNH 10687	Karschiidae	Karschia mastigofera Birula 1890
Mel	AMNH 10737	Melanoblossidae	Melanoblossia braunsi Purcell 1903
Mul	CAS 9033889	Mummuciidae	Munmucia sp.
Rh1	AMNH 2293	Rhagodidae	Rhagodes melanus (Olivier 1807)
So1	AMNH 7569	Solpugidae	Zeria sericea (Pocock 1897)

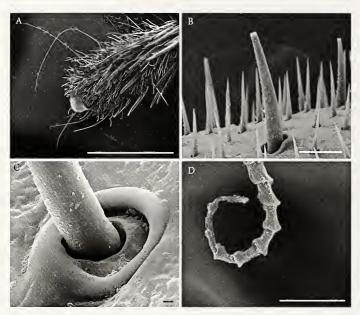


Figure 1.—Pedipalp segments and structures that were photographed. A) Tarsal segment of *Rhagodes melanus* (Rhagodidae), B) seta on femur of *Trichotoma michaelseni* (Gylippidae), C) base of seta on metatarsus of *Eremobates pallipes* (Eremobatidae), D) tip of seta on metatarsus of *Ceroma inerme* (Ceromidae). Scale line $A=3\,\mathrm{mm}$, scale line $B=50\,\mu\mathrm{m}$, scale lines C & $D=2\,\mu\mathrm{m}$.

We found 13 distinguishable setal types, mostly defined by the shape of the shaft and tip (Figs. 2A–M, Table 2). All sensory setae emerged from morphologically similar sunken pits. Thus, we mostly show only the tips and parts of the shafts of the different setae in Fig. 2. Table 2 summarizes how common each type of seta is among the 12 families. The bifurcated seta (Fig. 2A) and tapered tip seta (Fig. 2K) are common to all families. Some families such as Ceromidae (*Ceroma incrme* Purcell 1899) and Daesiidae [*Biton browni*

(Lawrence 1965)] seem to have unique setae (Figs. 2F, H, I). The nozzle tip seta (Fig. 2F), one type of truncated tip seta (Fig. 2M), and the tapered tip seta (Fig. 2K) all have terminal pores; thus, we suspect these function as chemoreceptors. Arthropod chemoreceptors can generally be distinguished by an apical pore (Slifer 1970; Foelix 1970; Foelix & Chu-Wang 1972; Harris & Mill 1973; Zacharuk 1980; Barth 2001; Talarico et al. 2006). The other setal types lack pores and may function as mechanoreceptors or as other types of sensory receptors (e.g., hygroreceptors, thermoreceptors, proprioreceptors).

The other structures found on the surface of the pedipalps may have some phylogenetic importance. Hexisopodidae (Chelypus barberi Purcell 1902) was the only family with spines on the tarsus, although additional species from the various families must be examined. Dorsal tarsal pores were seen in all 12 species representing the 12 families (Figs. 3A-D). These structures have been previously described (Bertkau 1892; Bauchhenss 1983). They occur in fields, containing a few to dozens of pores within a field along the dorsal surface of the tarsi (Figs. 3A, C). The shape of these fields may be phylogenetically informative; e.g., in Galeodes olivieri (Galeodidae), the field of pores extends diagonally from the proximo-medial surface of the tarsus, across the dorsal surface, ending at the anterio-lateral surface of the segment (Bauchhenss 1983 and Fig. 3A), whereas in C. barberi the pores are in two parallel groups down the dorsal surface of the tarsus (Fig. 3C). There were fewer tarsal pores on the pedipalps of Mummucia sp. (Mummuciidae) and Melanoblossia brannsi Purcell 1903 (Melanoblossidae). Bauchhenss (1983) suggested that reduction in the number of pores may be an adaptation against water loss through evaporation, although this hypothesis will have to be tested. These pores are hypothesized to be homologous to Blumenthal's tarsal organs in Araneae, which are hypothesized to function as olfactory organs (Bauchhenss 1983; Foelix 2011). Metatarsal pits were very apparent on the pedipalps of C. barberi (Figs. 3E–F); however, further analysis is required to determine the possible function of these structures. The concave shapes of the pits are noticeable enough to suggest that they may be of sensory importance. The pits are distributed evenly around the surface of the metatarsus (Fig. 3E).

This preliminary study of the sensory setae and structures on the pedipalps of Solifugae has revealed structures common to all families

Table	2.—	-Distrib	ution	of	setae	among	the	twelve	families.

Setal types	Ammo- trechidae	Cero- midae	Daes- iidae	Eremo- batidae	Gale- odidae	Gylip- pidae	Hexiso- podidae	Karsch- iidae	Melano- blossidae	Mummuc- iidae	Rhagod- idae	Solpug- idae
Bifurcated	X	X	X	X	X	X	X	X	X	X	X	X
Blunt		X		X	X	X	X	X	X	X	X	
Cavitate baton					X							
Clubbed				X					X	X		X
Imbricate		X	X	X	X	X	X		X	X		
Nozzle		X										
Papillae				X				X				X
Polymicro- digitus												
annulus			X									
Polymicro- digitus												
imbricate			X									
Simple		X	X									
Tapered	X	X	X	X	X	X	X	X	X	X	X	X
Truncated	X	X	X	X	X	X	Λ	71	21	X	7.	X
Truncated	Λ	Λ	Λ	Λ	Α	Λ				Λ		24
with porc	X											
Metatarsal pits							X					
Tarsal pores	X	X	X	X	X	X	X	X	X	X	X	X

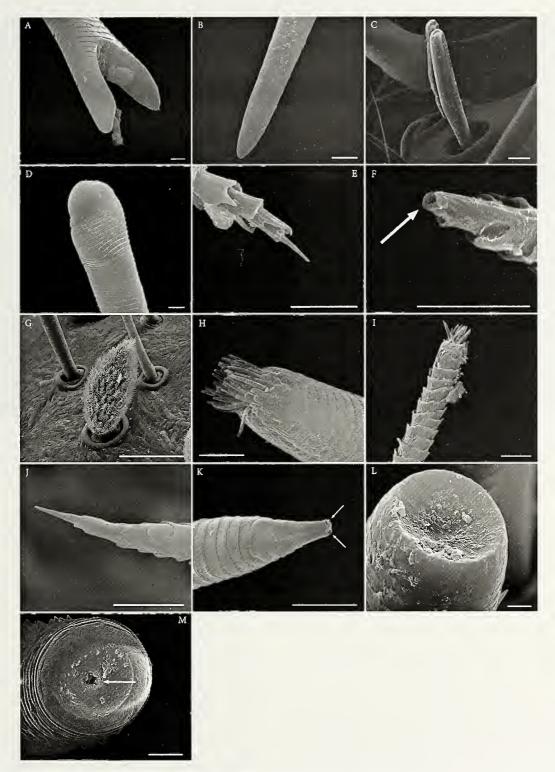


Figure 2.—Setal types found on the pedipalps of solifuges. A) Bifurcated seta tip on tarsus of *Ceroma incruue* (Ceromidae), B) blunt seta tip on the tibia of *Trichotoma michaelseni* (Gylippidae), C) cavitate baton seta on metatarsus on *Galeodes olivicri* (Galeodidae), D) clubbed seta tip on tarsus of *Eremobates pallipes* (Eremobatidae), E) imbricate seta tip on tibia of *C. inerme*, F) nozzle seta tip on femur of *C. inerme* (arrow points to pore), G) papilla on metatarsus of *E. pallipes*, H) polymicrodigitus (annulus) seta tip on femur of *Biton browni* (Daesiidae), I) polymicrodigitus (imbricate) seta tip on femur of *B. browni*, J) simple seta tip on femur of *E. pallipes*, K) tapered seta tip on tarsus of *B. browni*, L) truncated seta tip on tarsus of *T. michaelseni* with no pore evident, M) truncated seta tip on tarsus of *Brauchia angustus* (Ammotrechidae) (arrow points to pore). All scale lines = 2 μm except G = 50 μm.

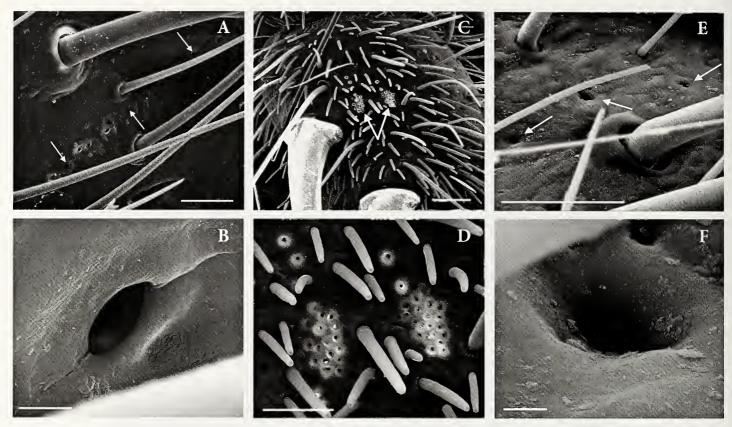


Figure 3.—Other structures found on the pedipalps of solifuges. A) Dorsal tarsal pore field of *Galeodes olivieri* (Galeodidae) (arrows point to pores), B) single dorsal tarsal pore of *G. olivieri*, C) tarsus with parallel dorsal tarsal pore fields of *Chelypus barberi* (Hexisopodidae) (arrows point to pore fields), D) dorsal tarsal pore field of *C. barberi*. E) distribution of metatarsal pits of *C. barberi* (arrows point to pits), F) metatarsal pit of *C. barberi*. Scale lines B & F = 2 μm; scale lines A, E, & D = 50 μm; scale line C = 100 μm.

such as the dorsal tarsal pores. We have also identified setae common to all families and setae that may be unique to individual families. Additional taxa within each of the 12 families must be examined in the future to verify the apparent phylogenetic usefulness of these sensory structures. In addition, in order to determine the function of the different types of setae, three experiments should be performed: 1) electrophysiology to detect mechano-, chemo-, hygro-, thermo-, and olfactory reception; 2) histological analysis to map out dendritic placement and help confirm function; and 3) behavioral studies to analyze setal function in their environment. Nevertheless, the present study verifies that the pedipalps of solifuges do play a major role in sensory perception.

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Thanks to USGS and Heather Lowers for the use of their SEM. Thanks to Lorenzo Prendini (AMNH), Charles Griswold (CAS), and Tharina Bird (SMN) for the loan and use of specimens. Thanks also to two anonymous reviewers for their helpful comments. This study was supported by National Science Foundation grants DEB-0346378 and DEB-1112263 awarded to Paula E. Cushing and DEB-0228699 awarded to Lorenzo Prendini.

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SHORT COMMUNICATION

The species referred to as Eurypelma californicum (Theraphosidae) in more than 100 publications is likely to be Aphonopelma hentzi

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Abstract. Despite the fact that taxonomically the name *Eurypelma californicum* (Ausserer 1871) has been regarded as a *nomen dubium* and thus invalid for several decades, it is increasingly used in non-taxonomic publications and on the internet. This makes it necessary to trace back the identity of the spiders involved. The taxonomy of *Eurypelma californicum* and *Aphonopelma hentzi* (Girard 1852) was investigated, and it is concluded that the spiders referred to as *Eurypelma californicum* in physiological publications of the last 35 years belong to an *Aphonopelma* species, most likely *A. hentzi*.

Keywords: Taxonomy, uomen dubium, synonymy, physiological research

Due to its size, longevity and easy maintenance, the North American theraphosid Eurypelma californicum (Ausserer 1871) has been used as a spider model to investigate the structure and function of hemocyanin (e.g., Schartau et al. 1983) and the circulatory system, including hemolymph and book lungs, venom biochemistry (e.g., Savel-Niemann & Roth 1989), and water and temperature-dependent aspects of physiological adaptations. The Web of Knowledge (online at http://webofknowledge.com) lists 139 publications (search term "Eurypelma californicum", accessed 22 July 2011) between 1977 and 2011, which have been cited more than 3200 times in more than 1500 other articles (Fig. 1). The most cited publications are reviews on hemocyanin by Van Holde & Miller (1995) and by Magnus et al. (1994), which were cited 263 and 259 times, respectively. A search on Google (accessed 22 July 2011) resulted in 4610 hits for the term "Eurypelma californicum". This indicates the importance of Eurypelina californicum and the results obtained by studying these spiders for science.

Unfortunately, today there is no valid species named *Eurypelma californicum*, which is a *nomeu dubium*, a name of doubtful origin that cannot be traced back to a valid species. Certain scientists, mainly physiologists and molecular biologists, however, increasingly eontinue using this invalid name, and so we face the fascinating question as to which species these publications might actually relate.

Eurypelma californicum was first described by Ausserer (1871). In his species description, however, he referred to Dolcschall (1852), which is an unpublished manuscript that includes a description of a female of this species as Theraphosa californica. According to Article 8 of the International Code of Zoological Nomenclature (ICZN 1999), Doleschall's (1852) manuscript does not represent a valid species description and, therefore, Ausserer is the author of this species. Nevertheless, Ausserer referred to Doleschall's manuscript and consequently named the spider Eurypelma californica Dol. Ausserer's species description was probably copied from Doleschall, as he placed it in citation marks and ends with "(trk.) Dol.", which probably means "transkribit (Latin, copied) from Doleschall". Although Ausserer called this species Eurypelma californica, most subsequent authors modified the name to Eurypelma californicum, which is grammatically correct (Eurypelma, Greek, neuter, means "broad foot").

The species description is as follows (translated from Latin): "brown, long cephalothorax, approximately parallel margins, head area well separated, strong and large chelicerae, considerably bent, hind part of the abdomen broader, slim legs of medium length, with broad tarsi. Length 15", length of the legs 4, 1, 2, 3." The second part (translated from German) is: "The prosoma is longer than broad, very elevated,

nearly parallel lateral margins, only in the back third broader, with short woolly hairs. The head is high, dorsal pit deep, transverse. Eye hump flat, limited by a furrow. Thick chelicerae, strongly bent, half the length of the prosoma, very hairy at the end and at the inner edge of the first segment. Thorax narrow, elongate. Opisthosoma as long as prosoma, broader towards the end; spinnerets bulkily elongated. Legs thin, short, woolly haired, with broad tarsi. Uniformly brown, venter darker, black to brown. California."

By modern standards Ausserer's description is poor and fits too many theraphosid species, although it was typical for its time. He did not provide any illustrations and did not mention a type specimen. It is also unclear whether he actually saw any specimens of this species. A further mistake happened when Ausserer attributed Doleschall's species to the genus Eurypelma, which had been erected by C.L. Koch (1850) for Mygale avicularia C.L. Koch 1842. Koch (1850) provided the first more or less useful description of this species and undoubtedly had examined some specimens. Due to the poor quality of earlier descriptions for Mygale and Theraphosa species from Linnaeus, Walckenaer and Hahn, their identity was very difficult to assess. Ausserer thus assumed that Koch misidentified Mygale avicularia and described it as Eurypelma rubropilosa Ausserer 1871. He also provided a (poor) definition of this genus, which was characterized (among others) by two spurs on the first tibiae of the male (Ausserer 1871). Simon (1892) assigned Eurypelma rubropilosa as the type species of Eurypelma. A few years later, Pocock (1901) detected in Koch's original description of Mygale avicularia the presence of only one male tibial spur. So the descriptions of Mygale avicularia and of Eurypelma rubropilosa and the transfer to Eurypelma excluded each other. Therefore, Pocock called Eurypelma a "genus ignotum at all events for the time being".

Petrunkevitch (1939a) pointed out that Ausserer's Eurypelma rubropilosa was not Koch's Eurypelma avicularia, whose identity was impossible to determine. Koch's genus description (prosoma more elongate, opisthosoma moderately thick, legs equally thick, whole body densely covered with long hairs, velvet brush of feet broad) was very broad, and today most theraphosids would fit into his genus Eurypelma. Since the type species is probably lost, a redeseription is impossible. Petrunkevitch concluded that "the genus Eurypelma must remain a genus incertum and invalidum". He proposed to transfer all Eurypelma species into other genera, where they best fit. Accordingly, Raven (1985) synonymized Eurypelma C. L. Koch 1850 with Avicularia Lamarck 1818 (see Platnick 2011).

For the identity of Eurypelma californicum this means that a species of which the type is lost, had been attributed to a doubtful genus.

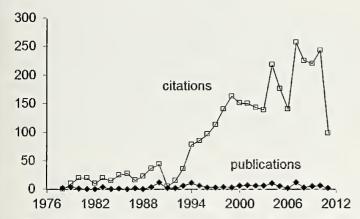


Figure 1.—Number of publications (black symbols, range 0–12 per year) and the number of citations (open symbols, range 0–257 per year) according to the search term "Eurypelma californicmu" in the Web of Knowledge. Data cover the period 1978–2011, but in 2011 only half a year is covered (accessed 22 July 2011).

Petrunkevitch first proposed a position in his new genus *Delopehua*, but later he transferred it into the genus *Dugesiella* (also misspelled as *Dugisiella*) (Petrunkevitch 1939a, b). Schmidt (1993) transferred *D. californica* to the genus *Aphonopehua*, but stated that it cannot be traced back to a valid species and, therefore, should be considered a *nomen dubium*, an opinion that is still accepted (Platnick 2011).

In his book on North American theraphosids, Smith (1995) described how he tried to locate the type of *Eurypelma californicum*. He could not detect it in major German museum collection lists and noted that the inadequate description by Ausserer makes it impossible to attribute this species to any known species or genus. Consequently, as no type specimen exists, and it seems to be impossible to positively identify any species from Ausserer's description, he also came to the conclusion that this name should be suspended.

From a taxonomic point of view, these analyses show that it is currently not possible to match a living species with the enigmatic specimen that Doleschall (1852) and Ausserer (1871) described more than 150 years ago. Unfortunately, the name Eurypelma californicum continues to be used in non-taxonomic publications and on the internet. This begs the question as to which valid species do the spiders belong that have erroneously been labeled as Eurypelma californicum over the past 50 years? I asked some research groups that had studied "Eurypelina californicum" for the origin of their spiders. It turned out that the spider stock at the University of Munich, from where most of this research originated, had been purchased from Carolina Biological Supply Company (Burlington, North Carolina, USA). Also many Eurypelma californicum spiders in other laboratories can be traced back to Munieh or to this company. On request, the company communicated that "the tarantula species we were shipping in the 1980's were Brachypelina smithi (Mexican redknee) and Aphonopelina hentzi (Texas Brown)". The origin of these spiders (Texas) and a few pictures available from publications and researchers clearly indicate that the species in question is likely to be Aphonopelma hentzi (Girard 1852), which is commonly known as the Oklahoma brown or the Texas brown.

In recent years, several investigations were performed to analyze the taxonomy and distribution of this species. Smith (1995) summarized the taxonomic situation, discussed the identity of *Aphonopelma hentzi* in detail, designed a neotype and described, for the first time, both sexes following modern standards. He also gave valuable information on the kind of habitat where this species occurs and further ecological data. Later, Murray (2006) and Hamilton (2009) carried out comprehensive analyses on species boundaries and their geographic distribution in the *Aphonopelma hentzi* complex and the separation of neighboring taxa. Based on their morphological and molecular assessments, both Murray (2006) and Hamilton (2009)

concluded that the distribution of *Aphonopelma heutzi* comprised large parts of Colorado and New Mexico, Oklahoma, southern Kansas, southern Missouri, Arkansas, northern Louisiana, and a major part of Texas.

The problem inherent to these findings is that Aphonopelma hentzi is apparently a wide-ranging species and it shows considerable morphological variation. Geographically, it overlaps with type locations and distribution areas of other Aphonopelma species (A. anax (Chamberlin 1940), A. armada (Chamberlin 1940), A. underatum (Chamberlin & Ivie 1939), as well as some potentially undescribed species). Whether these species are valid species or synonyms needs to be elarified in subsequent investigations.

In conclusion, it is clear that the bulk of physiological, biochemical, toxinological and molecular biological publications on *Eurypelma californicum* refers to an *Aphonopelma* species, most likely to be *A. hentzi*. It is highly recommended to keep voucher material (if possible, one well preserved male and female as well as tissue stored for DNA analysis), to specify the geographic origin of the specimens used, and to mention this in future publications. Once the separation of closely related *Aphonopelma* species has been completed and published, a suitable identification key should become available to properly confirm the species identity. "*Eurypehua californicum*", however, remains a *nomen dubium* and should not be used.

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SHORT COMMUNICATION

Clubiona analis Thorell 1895 from Burma: redescription and systematic position (Araneae: Clubionidae)

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Abstract. The holotype female of *Clubiona analis* Thorell 1895 is examined, illustrated, and redescribed. The systematic position of the species is discussed, and a map with the type locality together with those of related species is provided.

Keywords: Taxonomy, sac spider, Southeast Asia, Double Island

Recently, Southeast Asian representatives of the genus Clubiona Latreille 1804 have been revised by Deeleman-Reinhold (2001). Subsequently Dankittipakul & Singtripop (2008a, b), Jäger & Dankittipakul (2010) and Ono (2009) described new species from Thailand, Laos and Vietnam. The female of Clubiona analis Thorell 1895 was illustrated by Gravely (1931: Fig. 16C), Tikader & Biswas (1981: Figs. 118-119) and Biswas & Raychaudhuri (1996: Figs. 1, 6). None of these illustrations of C. analis is considered suitable for recognising the species unambiguously. Illustrations in the two latter publications even suggest that the authors misidentified or confused the species, as there are no similarities with real structures of the female type specimen; e.g., the bilobal posterior epigynal margin. Moreover, cheliceral teeth (3 anterior and 2 posterior) and eye arrangement (AME separated by same distance as PME) (Biswas & Raychaudhuri 1996: Figs. 1, 2) point clearly to a different species. Therefore, the present paper provides a redescription of C. analis and discusses its relationships.

The holotype female is preserved in 70% denatured ethanol. Female copulatory organs had already been dissected and were observed in 96% lactic acid. Spines of the prolateral, dorsal, retrolateral and ventral side of each leg segment are noted separately with three positions distinguished: proximal, medial, and distal. Some stronger bristles on dorsal patellae (d101 = 1 proximal and 1 distal bristle) may be counted as thin spines in other publications (e.g., Deeleman-Reinhold 2001). These are not listed in the spination pattern in the description below.

Abbreviations: ALE – anterior lateral eyes; AME – anterior median eyes; AW – anterior width of dorsal shield of prosoma; d – dorsal; OL – opisthosoma length; OW – opisthosoma width; p – prolateral; PL – prosoma length; PLE – posterior lateral eyes; PME – posterior median eyes; PW – prosoma width; r – retrolateral; RTA – retrolateral tibial apophysis; v – ventral; I–IV – referrring to leg numbers.

Museum collections (with curators): MHNG = Museum d'Histoire Naturelle Geneve, Switzerland (Peter Schwendiger), NHM = Natural History Museum London, England (Janet Beccaloni), SMF = Senckenberg Research Institute Frankfurt, Germany (Peter Jäger).

The author is grateful for comments by Vladimir Ovtsharenko (New York), an anonymous referee and Ingi Agnarsson, which helped to improve the mansucript.

Clubionidae Wagner 1887 Clubioninae Wagner 1887 Clubiona Latreille 1804

This genus currently consists of more than 460 species (Platnick 2011). Deeleman-Reinhold (2001) included a distinction of species groups within the genus. She did not follow Mikhailov (1995) in using subgenera, but used his intrageneric grouping, which is also used here.

C. analis, together with 33 other Clubiona spp., was listed as species incertae sedis in Deeleman-Reinhold (2001).

lystrix group Clubiona analis Thorell 1895 Figs. 1–10

Type material.—Female holotype (NHM 1895.9.21.72). BURMA [MYANMAR]: *Moulmein*: Double Island, Oates leg.

Note.—The type locality is located south of the Moulmein river entrance, ca. 25 air km south of Kyaikkami and 11 km offshore and is a granitic island (data from Rowlett 2010). GPS data obtained from Google Earth: 15°52′26.51″N, 97°35′09.56″E. The type is in bad condition, with almost all appendages and opisthosoma broken off. Only some measurements could be taken due to its fragile condition.

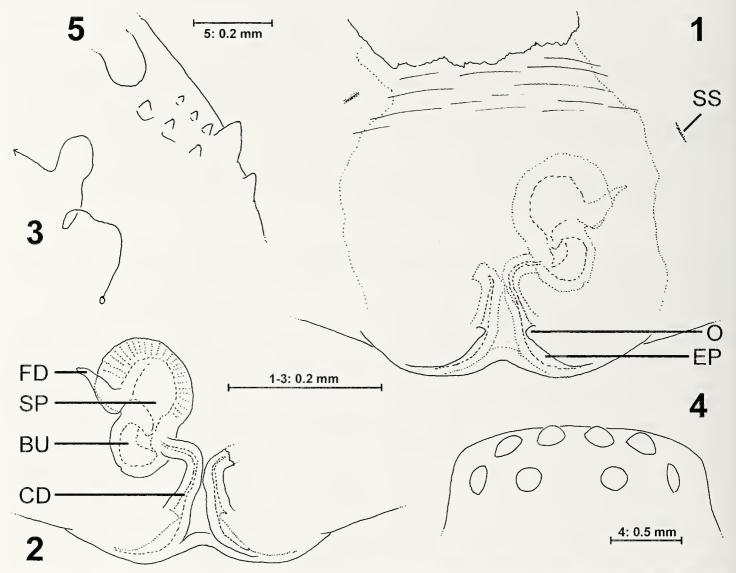
Additional material from *hystrix*-group examined for comparison.— Clubiona damirkovaci Deeleman-Reinhold 2001: 1 male, 1 female paratype (SMF 60487). MALAYSIA: *Malay Peninsula*: Gombak Research Station N of Kuala Lumpur, from bamboo internodes, D. Kovac leg. 1.VII.1991.

Clubiona maipai Jäger & Dankittipakul 2010: Male holotype, 9 male and 9 female paratypes (SMF, MHNG). THAILAND: Mae Hong Son Province: close to Ban Nam Rin, logged bamboo, by hand, D. Kovac leg. 13–24 September 2003.

Clubiona kuu Jäger & Dankittipakul 2010: Male holotype (SMF). LAOS (L15): Luang Prabang Province: SE Luang Prabang, Nam Khan, Ban Keng Koung, 372 m altitude, N 19°40.963'N, 102°18.442'E, along stream, disturbed forest, cultivated land, at tree bark, by hand, at night, P. Jäger & J. Altmann leg. 8.III.2006. 1 male paratype (SMF), Laos (L7), Luang Prabang Province, Nam Ou, Nong Khiao, Tham Pathok, 373 m altitude, 20°33.082'N, 102° 37.925'E, in front of cave, bananas, trees, bushes, at night, by hand, P. Jäger leg. 29 February 2008.

Diagnosis.—Small Clubioninae with body length of female holotype 9.2 mm, belonging to the *liystrix* species-group. Similar to *Clubiona damirkovaci* Deeleman-Reinhold 2001. Females can be distinguished from those of *C. damirkovaci* by the distinctly smaller bursae of the internal duct system (Fig. 2), by the more diagonally orientated epigynal pockets (Fig. 1), and the distinctly bilobal posterior epigynal margin (Fig. 1). Both species are distinguished from *C. maipai* by their short copulatory ducts (Fig. 2).

Redescription of female (holotype).—PL 4.1, PW 2.7, AW 1.8, OL 5.1, OW 2.0. Eye diameters (Fig. 4): AME 0.21, ALE 0.23, PME 0.18, PLE 0.20. Eye interdistances: AME-AME 0.13, AME-ALE 0.07, PME-PME 0.43, PME-PLE 0.22, AME-PME 0.16, ALE-PLE 0.12, clypeus AME 0.10, clypeus ALE 0.13. Leg measurements: leg I - (3.1, 1.8, 3.0, 2.0, -), leg II -; leg III - (2.5, 1.2, -, -, -); leg IV - (3.8, 1.6, -, -, -). Spination: Femur I p011, d111, r111, II -, III p111, d111, r111, IV p011, d111, r011; Patella I, III-IV r010; Tibia I v220.



Figures 1–5.—Clubiona analis Thorell 1895, holotype female from Burma, Double Island (right half of internal duct system damaged and omitted here). 1. Epigyne, ventral; 2. Vulva, dorsal; 3. Schematic course of internal duct system, dorsal (open circle – copulatory orifice, arrow – fertilization duct in direction of the uterus externus.); 4. Eye arrangement, dorsal; 5. Right cheliceral furrow, ventral. Abbreviations: BU – bursa copulatrix, CD – copulatory duct, EP – epigynal pockets, FD – fertilization duct, SP – spermathecae, SS – slit sensilla.

Chelicerae with weak frontal bulge (Figs. 8, 9), cheliceral furrow with 4–5 anterior (2 large proximal, 2–3 small distal) and 3 small posterior teeth (Fig. 5). Spinnerets and anal tuberele elongated (Figs. 6, 7).

Copulatory organ: As in diagnosis. Epigynal field as long as wide, with two slit sense organs antero-laterally. Copulatory openings situated medially in posterior half, accompanied by slightly semicircular pockets. Bilobal posterior margin extending slightly beyond epigastric furrow (Fig. 1). Copulatory ducts short, running first slightly converging in anterior direction, then bending laterally and leading into less sclerotised bursae. The latter broadly connected to sclerotised and thick-walled spermathecae. Fertilization ducts arising laterally, pointing antero-laterally (Fig. 2). A glandular appendage as present in C. damirkovaci (Decleman-Reinhold 2001: Fig. 23) or C. maipai (Jäger & Dankittipakul 2010: Figs. 42–44) could not be observed (Fig. 3). The bad condition of the holotype did not allow further treatment, therefore only a preliminary course of the internal duct system can be provided.

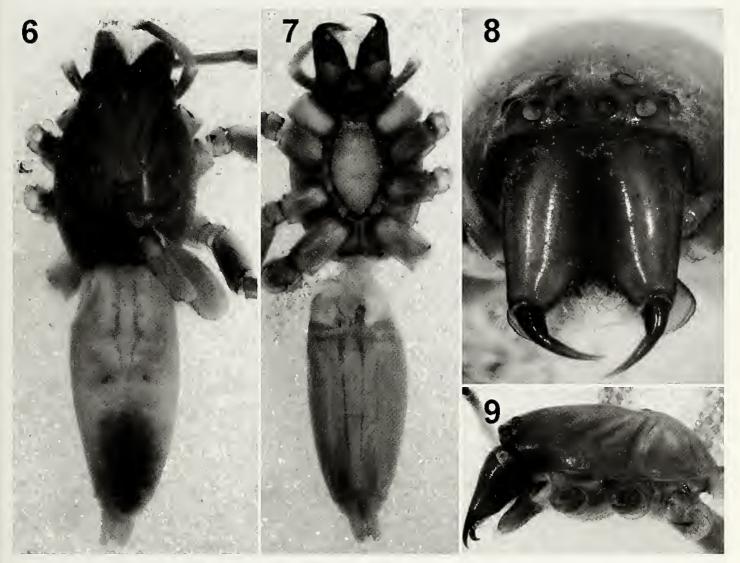
Coloration in ethanol: The bad condition of the holotype allows almost no statements about the real coloration. Prosoma and legs

seem to be reddish brown, and darker than opisthosoma. Labium with distinct and broad bright lip distally. Dorsal opisthosoma with one pair of muscle sigilla in the middle and an extensive dark patch in posterior half (Figs. 6–9).

Male: unknown.

Distribution.—Known only from the type locality (Fig. 10). Although the species may have a wider range, the records cited by Platnick (2011) are insufficient to establish the range of the taxon (see introduction; localities concerned: India: Calcutta, Dhakuria; Bangladesh: Bagerhat, Barisal, Dhaka, Jhenidah, Jessore, Khulna, Rajshahi).

Relationships.—Because of the posteriorly situated copulatory openings and the unique course and shape of the internal duct system, *C. analis* is clearly placed in the *liystrix*-group. It differs from other *liystrix* group species in having its orifices not hidden by a hood (Fig. 1), as described in the diagnosis in Deeleman-Reinhold (2001:101). The epigynal pockets accompanying the openings are, again, similar to typical *liystrix*-group representatives. *D. damirkovaci* is most similar referring to the structure of its female copulatory organs, but there are more species to be considered; e.g., described or



Figures 6-9.—Clubiona analis Thorell 1895, holotype female from Burma, Double Island, habitus (6 dorsal; 7 ventral; 8 prosoma, frontal; 9 prosoma, lateral).

listed in Chrysanthus (1967) from New Guinea (C. ericius, C. ineraukensis). C. maipai (known from both male and female) and C. kuu (only known from the male) also seems related according to the similarity of the male copulatory organ of both species to that of C. damirkovaci. Fresh material from both sexes of C. analis would help to illuminate relationships within the *hystrix* group.

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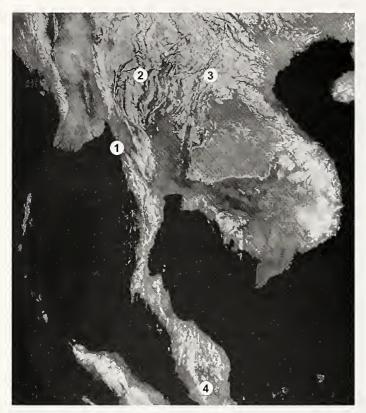


Figure 10.—Type localities of representatives of the *Chubiona hystrix*-group: 1. *Chubiona analis* Thorell 1895, Burma, Double Island; 2. *Chubiona maipai* Jäger & Dankittipakul 2010, Thailand, Mae Hong Son Provinee, Ban Nam Rin; 3. *Chubiona kuu* Jäger & Dankittipakul 2010, Laos, Luang Prabang Province, Ban Keng Koung; 4. *Chubiona daunirkovaci* Deeleman-Reinhold 2001, Malaysia, Gombak Research Station.

SHORT COMMUNICATION

Maternal care in the Neotropical harvestman Liogonyleptoides tetracanthus (Opiliones: Gonyleptidae)

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Abstract. We describe post-ovipositional maternal care in *Liogonyleptoides tetracanthus* Mello-Leitão 1932 (Gonyleptinae) and experimentally evaluate the protective role of this behavior against egg predation under laboratory conditions. Females laid 138.8 eggs on average and remained close to the clutch during the entire day. Eggs hatched after 11–15 days and nymphs dispersed from maternal protection after one to two days. Most of the experimental clutches left unattended were entirely consumed by conspecifics in 2–3 days. There was no reduction in egg number in the clutches protected by females. Although biological data are scarce, there are cases of egg hiding, paternal and maternal care within the subfamily Gonyleptinae. This diversity of forms of parental care is unusual when compared to other gonyleptid subfamilies, and future systematic revisions of the polyphyletic Gonyleptinae should include parental care as a potential source of phylogenetic information.

Keywords: Egg-guarding, cannibalism, evolution, Gonyleptinae, post-ovipositional parental care

Many species of harvestmen in the suborder Laniatores exhibit post-ovipositional parental care (Machado & Raimundo 2001). Maternal care, in particular, is widespread among Neotropical representatives of the superfamily Gonyleptoidea, in which female egg-guarding has evolved at least seven times independently: once in the stygnopsid Hoplobunus boneti (Goodnight & Goodnight 1942), at least once in the closely related cranaid genera *Phareicranaus* Roewer 1913 and Santinezia Roewer 1923, once in the cosmetid Erginulus clavotibilialis (Pickard-Cambridge 1905), once in the gonyleptid Neosadocus maximus (Giltay 1928), at least once in the Pachylinae (Gonyleptidae), once in the ancestor of the Bourguyiinae (Gonyleptidae), and once in the ancestor of the Goniosomatinae (Gonyleptidae) (see references in Machado & Macías-Ordóñez 2007 and Hunter et al. 2007). Here we provide additional observation of maternal care in the previously unstudied genus Liogonyleptoides Mello-Leitão 1925 (Gonyleptidae) and experimentally evaluate the protective role of maternal care against egg predation under laboratory conditions.

We collected 24 individuals of L. tetracanthus (Mello-Leitão 1932) at the edge of an Atlantic Forest fragment near the city of Sooretama, state of Espírito Santo, south-eastern Brazil. We brought the individuals to our laboratory at the Universidade de São Paulo, São Paulo, Brazil, and placed them in two terraria (40×90 cm base, 20 cm height) containing soil, rocks and small pieces of wood. Each terrarium received 12 individuals (four males and eight females) that were individually marked on their dorsal scute with colored dots of enamel paint and fed ad libitum with canned dog food. Climatic conditions in the laboratory were: 21-24° C, 61-80% rel. humidity, and 12:12 h L:D cycle (lights on at 06:00 h). We conducted behavioral observations at 1-6 day intervals in January-April 2010. Behavioral samplings consisted of scans conducted three times a day: morning (09:00-11:00 h), afternoon (13:00-14:00 h), and night (20:00-23:00 h). In each scan ("point sampling" sensu Martin & Bateson 1994), we recorded: a) the presence of clutches in the terraria, b) the presence of a guarding female close to each clutch, and c) the presence and behavior of any other individual near each clutch. We also made continuous records (sensu Martin & Bateson 1994) of all relevant behavioral observations, such as agonistic interactions between individuals, ovipositions, and egg predation events. Continuous

records lasted from 1 to nearly 30 min, depending on the duration of the behaviors we were observing. In addition, we photographed half of the 12 clutches 3–5 days after oviposition and used these photographs to count the eggs. In the remaining cases, eggs were laid deep inside cavities of rocks and wood so that it was not possible to photograph or to count them.

We evaluated the protective role of maternal care against egg predation through an experiment in which we removed five guarding females from their clutches between January and March. Two females were removed from one terrarium (one on January 21st and the other on March 2nd), and three were removed from the other also on different dates (on February 25th, March 8th and 25th). At the moment we removed these females, at least two other females in each terrarium were also guarding eggs, and we used those females as controls in our experiment. The clutches from which we removed the females were 4-5 days old, and we monitored them daily for 6 days or until all eggs were consumed. We also monitored the control clutches and counted the total number of eggs in each of them after 6 days of experiment. At the end of April, when the experiment was already finished, several individuals (males and females) died due to unknown reasons in both terraria, and we resumed our observations. We deposited voucher specimens of males and females in the arachnological collection of the Museu de Zoologia da Universidade de São Paulo (MZSP), state of São Paulo, Brazil.

Twelve of 16 L. tetracanthus females laid eggs in the laboratory during the four months of observations. Eggs were laid in single large clutches inside cavities of rocks (n = 9) and wood (n = 3), and females did not cover the eggs with debris or mucus, but remained guarding them (Fig. 1). The clutches had 138.8 \pm 59.6 eggs (mean \pm SD, n=6clutches), which were light cream in color when recently laid (Fig. 1), but darkened during embryonic development. During most of the day, guarding females stayed prostrate on the eggs or at the side of their clutches with legs II or IV touching the eggs (96% of 149 scans; Fig. 1). We observed guarding females away from their clutches in only three occasions. In all these cases, they left the eggs unattended to search for food at night. Embryonic development lasted 11-15 days. After egg hatching, guarding females remained with the hatched nymphs until they dispersed, which generally occurred one to two days after eclosion. Hatched nymphs did not feed while under maternal protection, but attacked and cannibalized other nymphs (including siblings) after dispersion. In one of the clutches there were

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Figure 1.—Guarding female of the harvestman *Liogonyleptoides* tetracantlms, prostrated close to a clutch of recently laid eggs.

four eggs infected by fungus that did not hatch. The guarding female remained on these infected eggs for four days before abandoning them.

Three of the five clutches from which we experimentally removed the guarding females were entirely consumed and two partially consumed by conspecifics in two to three days. We observed no reduction in the number of eggs in the seven clutches that were protected by females. During daylight hours, we observed non-parental individuals (males and females) prostrate near the protected clutches in 33.5% of the scans (n=149 scans), but we recorded no aggressive interactions by the guarding females. At night, when the individuals were more active, guarding females were generally aggressive when other individuals approached their eggs or nymphs (n=5 observations). In these situations, guarding females attacked the intruders with the pedipalps, sometimes grasping their legs and carrying them away from the offspring (n=2).

The oviposition sites used by *L. tetracantlms* females in our terraria are similar to those of the cosmetid *Erginnlus clavotibialis* (Goodnight & Goodnight 1976) and most species of Pachylinae exhibiting maternal care (Capocasale & Bruno-Trezza 1964; Juberthie & Muñoz-Cuevas 1971; Elpino-Campos et al. 2001). On the other hand, the lack of debris around the eggs, is a derived trait of Bourguyiinae (Machado & Oliveira 2002), Goniosomatinae (Machado 2002), and Gonyleptinae (Machado & Vidal 2001; this study). Unlike many gonyleptid species in which females care for the offspring, hatched nymphs of *L. tetracantlms* remain under parental protection for only a short period (no longer than five days). In representatives of Bourguyiinae and Goniosomatinae, for instance, newly hatched nymphs remain under maternal protection for up to 14 days (Gnaspini 1995; Machado & Oliveira 1998, 2002).

In species with maternal care, females lay larger eggs than species exhibiting no care (Machado & Raimundo 2001). Heavily yolked eggs may supply more reserves, allowing the young to develop to a larger size inside of the egg prior to hatch. Moreover, nymphs hatched from larger eggs may remain longer under maternal protection, consuming the remaining energetic reserves before dispersing to forage. Therefore, we hypothesize that interspecific differences in the time of nymph dispersion may be related to egg size, so that nymphs hatched from relatively small eggs are likely to disperse faster than nymphs hatched from relatively large eggs. Given that maternal care has evolved many times independently within the suborder Laniatores (Machado &

Macías-Ordóñez 2007; Hunter et al. 2007), this hypothesis can be tested in the future using a comparative approach.

Our captive experiment showed that female presence has an important protective role against predation in L. tetracanthus. Conspecifics, the only predators present in the terraria, consumed entire clutches within a few days. Similar results have already been described in the wild for a variety of neotropical harvestmen, including the gonyleptids Acutisoma longipes Roewer 1913, Bourgnyia trochanteralis (Roewer 1930), and Serracutisoma proximum (Mello-Leitão 1922) (see references in Buzatto et al. 2007). Eggs of L. tetracanthus may also be attacked by fungi and, although fungusinfected eggs did not develop, guarding females do not eat or remove such eggs from their clutches. Field experiments with A. longipes demonstrated that guarding females are also unable to protect eggs against fungal attack (Machado & Oliveira 1998). Only one harvestman species, the paternal Zygopachylus albomarginis Chamberlin 1925, is known to control egg fungal attack (Mora 1990). Although the ability to control fungus infection on the eggs is probably rare in harvestmen (Machado & Raimundo 2001), it is common in centipedes (Brunhuber 1970) and millipedes (Kudo et al. 2011) with parental care.

In its current systematic definition, the Gonyleptinae includes 142 species (Kury 2003), and three forms of parental care have been described for species of the subfamily. Females of Mischonyx cuspidatus (Pereira et al. 2004), Parampheres albimaculatus and P. ronae (Stanley 2011) hide their eggs on the soil, under fallen trunks, or among the leaf litter. Males of Neosadocus sp. and Gonyletes saprophilus guard eggs and early hatched nymphs (Machado et al. 2004). Lastly, Neosadocus maximus (Machado & Vidal 2001) and L. tetracanthus (this study) exhibit maternal care. Although biological data are scarce, such diversity of forms of parental care in the Gonyleptinae is unusual when compared to other subfamilies of Gonyleptidae. In most harvestmen, species belonging to the same genus or even the same subfamily exhibit the same form of parental care (e.g., Nazareth & Machado 2009). Since the Gonyleptinae is almost certainly a polyphyletic group (Kury & Pinto-da-Rocha 2007), and most of the genera are not correctly diagnosed and delineated, future systematic revisions should include the forms of parental care as a potential source of phylogenetic information. Therefore, additional information on the reproductive biology of a wider sample of species is extremely valuable for both behavioral and systematic studies.

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SHORT COMMUNICATION

Sexual receptivity varies according to female age in a Neotropical nuptial gift-giving spider

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Abstract. For many spiders, sex pheromones associated with female silk are important stimuli that elicit male searching and courtship behaviors. In that context, female sexual receptivity and chemical attractiveness can depend on age and reproductive status. In Paratrechalea ornata (Mello-Leitão 1943) (Araneae: Trechaleidae), males offer females a nuptial gift (a prey wrapped in silk) during courtship. Gift construction is elicited by the presence of female silk, and silk attractiveness is influenced by female age, increasing from 20 days after the female reaches adulthood. Our goal was to investigate whether female age affects female sexual receptivity and to discuss the relationship between receptivity and silk attractiveness. We exposed 26 virgin females, from 2 to 28 days after the final molt, to males offering a nuptial gift. Female sexual receptivity was age dependent and increased with adult female age. Females over 15 days from adulthood accepted more gifts than younger females, but the latency of female gift acceptance was not affected by female age. Female sexual receptivity is synchronized with chemical attractiveness, suggesting that females' pheromone release is adjusted at a particular mating age. We suggest that young virgin females may invest more in foraging and maturing gonads than in mating, accounting for the delay in receptivity and chemical attractiveness.

Keywords: Chemical attractiveness, age dependence, receptive females, Paratrechalea ornata

Reproduction occurring soon after sexual maturity can benefit individuals by increasing the number of offspring in a reproductive season and/or accelerating offspring reproduction (Moore & Moore 2001; Oli et al. 2002). However, these benefits may be balanced by associated costs such as reductions in growth, survival, or future reproduction (Stearns 1989). In fact, age can affect female fitness, and in some species it has been suggested that early mating is suboptimal for females (Krüger 2005; Maklakov et al. 2007). Consequently, young virgin females can sometimes delay first mating and reject males, for instance, when they are not physiologically mature (Markow 2000). However, resisting and rejecting behaviors may be costly for females due to male harassment (Rowe et al. 1994); females can decrease costs by being undetectable and avoiding direct contact with males (Wilcox 1984; Krupa et al. 1990). Sex pheromones, especially those presented in the silk (contact sex pheromones), are a very important communicatory channel associated with male attraction in spiders (Gaskett 2007). In some species, female sexual receptivity and chemical attractiveness depend on adult age and reproductive status (Roberts & Uetz 2005; Baruffaldi & Costa 2010). In this context, females may have the opportunity to maintain their crypticity when the costs of mating are high (Stoltz et al. 2007).

In the spider species Paratrechalea ornata (Mello-Leitão 1943) (Araneae: Trechaleidae), the male offers the female a prey wrapped in silk during courtship (Costa-Schmidt et al. 2008; Albo & Costa 2010). Since there is no report of gift stealing during courtship and before mating in this species, once the female accepts the nuptial gift by grasping it with her chelicerae, the male adopts the mating position and starts sperm transfer. Albo et al. (2009) reported age dependence in both male and female gift construction behaviors. Older males, exposed to female silk and/or female presence, are more prone to construct nuptial gifts than younger ones. Similarly, older females elicit more gift constructions than younger females, suggesting that female attractiveness is affected by female age.

If chemical attractiveness is linked to sexual receptivity, we predict

younger females to be less sexually receptive (more reluctant) to male

courtship than older ones. Our aim in this study is to elucidate the relationship between adult age and sexual receptivity by exposing P. ornata females of different ages to courting males, monitoring the occurrence and latency of female gift acceptance. We discuss the results, taking into account a previous study of female chemical attractiveness in P. ornata (Albo et al. 2009).

Paratrechalea ornata is a South American crepuscular/nocturnal and semi-aquatic spider (Carico 2005). In Uruguay, this species has two reproductive seasons (April-July and September-December), apparently with no overlap (Albo & Costa unpublished data). We collected 52 subadults during September and October 2008 in Yerbal Chico Stream (Quebrada de los Cuervos, Department of Treinta y Tres, 32°55′30.50″S, 54°27′33.10″W), Uruguay. To accelerate maturation we housed spiders in individual glass jars (8 cm high × 11 cm diameter) in a warm room at an average temperature of 26.6°C (± 1.1 SD). We measured age from date of sexual maturity (day of final molt). Male age averaged 37.4 days (\pm 32.3 SD), with no interaction with gift acceptance or female age ($X^2 = 0.03$, df = 1, P = 0.85; $X^2_{M^*F}$ = 2.36, df = 1, P = 0.12). After the spiders molted, we maintained them and carried out the experiments in the same room at 23.1°C (± 2.2 SD). Individuals were fed weekly with a mixed diet of termites (Nasutitermes sp.), fruit flies (Drosophila melanogaster) and pieces of mealworm (Tenebrio molitor). Water was provided daily with moist cotton wool.

We carried out the experiments after sunset in large glass cages (30 cm long, 15 cm wide, 20 cm high), each containing a layer of pebbles and a Petri dish with water, from 17 September to 26 December 2008. We exposed 26 virgin females, ranging in age from 2-28 days (after final molts), to males offering a nuptial gift. Males and females were fed two fruit flies (Drosophila melanogaster) on the day before the experiment to standardize feeding conditions before the experiment; afterwards females were transferred to experimental glass cages. Each experiment consisted of three steps. First, we placed the male in a Petri dish containing haphazard female silk, offering a large fruit fly (Drosophila funebris) and eliciting prey-wrapping behavior (Albo et al. 2009). Second, seven minutes after the last silk wrapping, we transferred the male with the gift to the experimental glass eage, where he made contact with the female silk but remained separated from the female by

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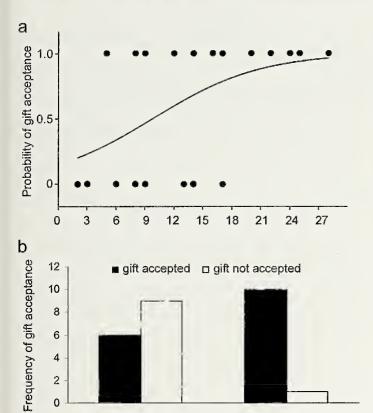


Figure 1.—Occurrence of female gift acceptance a) according to female age and b) before and after 15 days of adulthood.

Female age (days)

> 15

0 - 15

2

an opaque wall. Third, after five minutes for male habituation to the new environment, we removed the wall allowing a male-female encounter. The experiment concluded when the female accepted the gift or after female rejected the male. We recorded female gift acceptance when the female grasped the gift offered by the male, and female rejection of the male when the female ran away without grasping the gift. We registered both frequency and latency of female gift acceptance. Latency of gift acceptance was measured from the point of first encounter between the sexes to the point when the female grasped the gift with its chelicerae. Voucher males and females were deposited in the arachnological collection of the Facultad de Ciencias, Montevideo, Uruguay.

Data analysis was performed with PAST statistical package (Hammer et al. 2003). We tested normality of residuals and homogeneity of variances with Shapiro-Wilk and Levene tests, respectively. For comparing mean values, we used one-way ANOVA and the Student's t test for two independent samples. We used logistic regression to assess whether adult age affected gift acceptance and Fisher's exact probability test adjusted to multiple comparisons (Bonferroni correction) to compare frequencies of gift acceptance. The data were transformed whenever necessary to meet parametric assumptions.

We found that female gift acceptance varied according to female age. Female sexual receptivity increased correspondingly with the number of days after reaching adulthood ($X^2 = 7.45$, P = 0.006; Fig. 1a). When we analyzed the data in detail, we found that females more than 15 days past the final molt (older females) were more receptive and showed 90% gift acceptance, while females less than 15 days past the final molt (younger females) rejected males offering gifts more often (only 38% gift acceptance) (Fisher exact test, with Bonferroni correction: P = 0.02; Fig. 1b). Reluctant females rejected

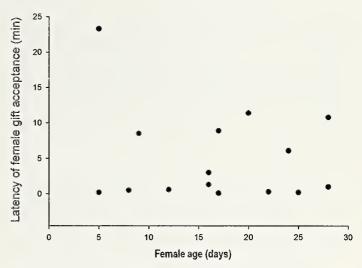


Figure 2.—Latency of gift acceptance (in minutes) according to female age (in days).

males by running away without grasping the gift, while receptive females stayed close to the courted male and grasped the gift.

We did not find statistical differences in the latency of gift acceptance either among different female ages ($F_{I,I3} = 0.001$, P =0.99; Fig. 2) or between the two female age categories, being 6.62 min (± 9.9 SD) in younger females and 4.31 min (± 4.5 SD) in older females (Student *t*-test: $t_{10.8} = 0.010$, P = 0.99). No qualitative differences were observed between female adult age and male courtship and/or gift offering behaviors.

Our findings show that female sexual receptivity and mate acceptance are influenced by female age, as was previously indicated in cockroaches and spiders (Moore & Moore 2001; Uetz & Norton 2007). Fifteen days after reaching adulthood, females accept mates more frequently, suggesting an optimal age for starting sexual activities. Adaptively and similar to young virgin females of many animal species, P. ornata females may be investing in other activities such as foraging and gonad maturation before starting investment in reproduction (Markow 2000; Bukowski & Avilés 2002; Krüger 2005). Maklakov et al. (2007) reported in a seed beetle that early matings are suboptimal for females, since females mating early in life suffer from a reduction in lifetime fecundity. Consequently, female attractiveness may be adaptively adjusted to minimize possible encounters with males early in their adult lives (Wilcox 1984; Krupa et al. 1990; Stoltz et al. 2007). For instance, Schulz & Toft (1993) and Schulz (2004) indicated that female spiders may control their attractiveness by varying their pheromone emissions. In the wolf spider Schizocosa malitiosa (Tullgren 1905), females reach their maximal chemical attractiveness 20 days after reaching adulthood (Baruffaldi & Costa 2010). Similarly, Albo et al. (2009) reported that the silk of mature P. ornata virgin females is more chemically attractive to males after 20 days of adulthood and elicits more frequent male gift construction than that of younger females.

Adult female P. ornata can live approximately 90 days in the field (Albo & Costa unpublished data). Hence, the difference in days between sexual receptivity (15 days after reaching sexual maturity) and chemical attractiveness (20 days after reaching sexual maturity) is only 6% of a female's adult lifespan. This suggests that pheromone release and female sexual attraction are adjusted to sexual maturity at a particular mating age. From the male perspective, finding young females, despite their low receptivity, may be beneficial. Because they are entelegyne spiders, being the first to mate with a female probably increases a male's percentage of paternity due to first male sperm priority (Austad 1984; but see Uhl 2000). In addition, females producing a suboptimally attractive pheromone could be indirectly

selecting good searcher males (Svensson 1996; Jaffe et al. 2007; Baruffaldi & Costa 2010), which may explain cases of female acceptance of males within 15 days of reaching maturity in this study.

In conclusion, it seems that female age, sexual receptivity, and chemical attractiveness are important factors influencing female reproductive decisions in *P. ornata*. We need further studies testing the effects of female age on reproductive success (i.e., copulation duration and number of fertilized eggs), allowing for the estimation of probable costs associated with early reproduction in this species.

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We are grateful to Alicia Postiglioni and Laura Montes de Oca for their assistance in field collections, to Laura Montes de Oca for help in spider maintenance, and to Macarena González for her collaboration in the experiments. We also thank Fernando G. Costa, Macarena González, Associate Editor Linden Higgins, and two anonymous reviewers for constructive comments on the manuscript, and Peter Schlanbusch for improving the English.

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SHORT COMMUNICATION

The effects of temperature on egg development and web site selection in Nephila clavipes

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Abstract. Temperature affects metabolic rate and egg development in mature female spiders. For temperate species, declining temperatures toward the end of the reproductive season may limit reproductive output, particularly for late-maturing females. Although spiders are known to alter their web-site preferences in response to temperature, it is unknown whether females can use web placement to overcome low temperatures that prohibit reproduction and thus extend their reproductive time frame. I surveyed web temperatures for female Nephila clavipes (Linneaus 1767) to compare female web sites and control sites at the beginning and end of the reproductive season in order to assess whether females change their web preferences in response to declining temperatures. Survey data showed that the web sites chosen by females at the end of the reproductive season have a higher minimum temperature than sites occupied during the early season. In a laboratory experiment, I addressed whether a low but biologically relevant temperature affects egg development and the female's ability to oviposit in N. clavipes. Females kept at 16° C failed to oviposit and showed signs of slowed egg development. Thus this preliminary study suggests that females may be able to protect themselves against temperatures that are prohibitively low for reproduction, but further experiments should explore the effects of temperature on egg development and web-site selection in this species.

Keywords: Behavioral thermoregulation, metabolism, seasonal constraints, size

A spider web is a microenvironment with a unique set of abiotic and biotic conditions that affect the survival and reproductive success of the web-owner (Agnarsson 2003; Rittschof & Ruggles 2010). Because web site choice is so critical to individual fitness, many researchers have examined the criteria that female spiders evaluate when choosing a web site (e.g., Elgar et al. 1996; Heiling 1999; Adams 2000; Bilde et al. 2002). Web temperature is one abiotic factor that is known to affect prey availability (Herberstein & Fleisch 2003), the web owner's metabolic rate and activity level (Lubin & Henschel 1990; Li & Jackson 1996), as well as web site selection in certain species (Henschel et al. 1992; Voss et al. 2007). In an environment where temperature is highly variable over time and space, adult females, whose egg development is temperature-dependent, will benefit if they can moderate their body temperature through web site selection. Although past studies show that female spiders have thermal preferences, few studies have attempted to address whether females can successfully overcome suboptimal temperatures through web site selection, and thus extend the time period during which they are reproductively active.

In the temperate (e.g., Florida) populations of the golden orb-web spider Nephila clavipes (Linneaus 1767) (Araneae, Nephilidae), low temperatures at the end of the season can result in female death prior to successful oviposition (Higgins 2000). Because individuals have only a single season to reproduce, the possibility that females can prolong their lifespan or reproductive period through web site placement has important fitness implications. I surveyed mean and minimum web temperature for females in the field at the beginning and end of the season to assess how female web

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placement affects body temperature and whether site preferences change toward the end of the season as temperatures decline. In addition, I performed a laboratory experiment to test the effects of late-season minimum temperatures on egg development in adult *N. clavipes*. Together these data lend support to the hypothesis that seasonal decline in temperature limits egg development and affects web site selection in *N. clavipes*. By changing web site preferences, females may be able to overcome late season reproductive constraints behaviorally.

I measured temperatures at female web sites early (July) and late (October) in the 2010 reproductive season. At both times of the season, I sampled the first 20 mature female webs found within 10 m of a 200 m linear transect in the Ordway-Swisher Biological Station. For each web site, I established a control site at a height of 80 cm and a distance of 10 m from the web site in a cardinal direction chosen at random. At both web and control sites, I sampled temperature every 5 min for 72 h using data loggers (described below) to determine mean and minimum temperatures. The 72 h time period is within the range of a typical web tenure for an adult N. clavipes in this population (Rittschof & Ruggles 2010). The 20 early season web and control sites were sampled in three blocks: 16-19 Jul 2010 (n = 7 webs and 7 controls), 21–24 Jul 2010 (n = 7 webs and 7 controls), and 27-30 Jul 2010 (n = 6 webs and 6 controls). Similarly, I sampled webs in the late season in two blocks: 5–8 Oct 2010 (n = 10 webs, 10 controls) and 10–13 Oct 2010 (n = 10 webs, 10 controls). Finally, in order to determine whether some web sites chosen in the early season become unsuitable in the late season due to temperature changes, I resampled the 20 early-season web sites (but not the controls) during the late season.

In order to measure web site temperature (a proxy for female body temperature) at a fine spatial scale over an

extended time period, I developed a physical model (Bakken 1992) for a female N. clavipes abdomen and embedded an iButton® temperature data logger (Maxim Integrated Products, Sunnyvale, CA) within the model. The model was a piece of brown clay (Van Aken International, Rancho Cucamonga, CA). After I embedded the data logger, the disc-shaped model was approximately 21 mm in diameter and 8 mm deep with the data logger embedded 3 mm in clay. I verified that the brown clay mimics temperature fluctuations in a female abdomen by comparing thermocouple measurements from the abdomen of a dead N. clavipes to a similar-sized piece of brown modeling clay over a 24 h period (5 min intervals). The temperature distributions were not significantly different across the sampling period (Kolmogorov-Smirnov Test, D = 0.027, P = 0.99).

When sampling web and control sites, I mounted each model onto a pole (1 cm in diameter), positioning the model within 5 cm of the dorsal side of the female spider at each web site. Using this method I could approximate the temperature changes experienced at the web hub, which is the resting position for female *N. clavipes* during the day and night. This approach, however, did not allow me to capture the effects of web or body orientation on body temperature, which are other means of behavioral thermoregulation in spiders (Herberstein & Heiling 2001; Ramirez et al. 2003). At control sites, I mounted data loggers on poles 80 cm from the ground.

First I tested whether web site selection is affected by thermal characteristics by comparing the mean and minimum temperatures at web and control sites using a nested ANOVA with sampling date nested within season. For this analysis, I omitted the late-season data from re-sampled early-season webs. There were significant effects of season and sampling date on mean web temperature. However, there was no evidence of temperature-based web site selection: there was no difference between web sites and control sites in either mean temperature (n = 20 webs and 20 control sites at two times of the season, Season: $F_1 = 1416.7$, P < 0.0001; Sampling date: $F_3 = 156.8$, P < 0.0001; web versus control site: $F_5 = 1.2$, P =0.48) or minimum temperature (n = 20 webs and 20 control sites at two times of the season, Season: $F_I = 11830.9$, P <0.0001; Sampling date: $F_3 = 926.5$, P < 0.0001; web versus control site: $F_5 = 1.2$, P = 0.33). Thus although web temperatures change on a daily basis and decrease as the season progresses, on a given day, female web temperatures do not differ from sites within the same area chosen at random. One explanation for the lack of significant temperature difference between web and control sites within a single season period could be that the 10-m difference in location between web and control sites (see above) failed to capture the range of temperature variation found along the sampling transect.

In order to test whether spiders select webs at the beginning and end of the season on the basis of temperature profile differences, I re-sampled the 20 early-season web sites simultaneously with the 20 web sites in the late season. There were no spiders occupying the early season web sites at the time that they were re-sampled in the late season. For this analysis I constructed an ANOVA with season (i.e., whether sites were occupied in the early or late season) nested within sample date. In this analysis, there was no significant effect of

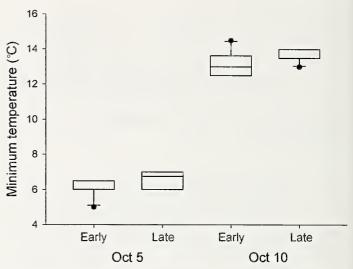


Figure 1.—Minimum web temperatures measured on either 5 Oct 2010 or 10 Oct 2010, comparing webs preferred in the early season (early, n=20 total) to webs preferred in the late season (late, n=20 total). The lower and upper sides of the boxes represent the $25^{\rm th}$ and $75^{\rm th}$ percentiles respectively, and horizontal lines through the middle of the boxes are the medians. Vertical lines extending from the bottom and top of the boxes show the $10^{\rm th}$ and $90^{\rm th}$ percentiles respectively, and the data points shown are values beyond the $10^{\rm th}$ and $90^{\rm th}$ percentiles.

season on mean temperature, but there was a significant effect of sample date (season: $F_2 = 2.7$, P = 0.083; sample date: $F_1 = 1064.8$, P < 0.0001). In addition, season had a significant effect on minimum temperature. Web sites occupied by females in the early season had lower minimum temperatures in the late season than sites occupied by females in the late season (mean_{early} = 9.6° C, SE = 0.8° C; mean_{late} = 10.1° C, SE = 0.8° C; sampling date: $F_1 = 2074$, P < 0.0001, season occupied: $F_2 = 1.19$, P = 0.0104, Fig. 1). These results suggest that females may respond to temperature when selecting web sites.

In addition to assessing field web site temperatures, in a laboratory experiment I examined the effects of temperature on egg development. I used ehanges in abdomen size to estimate egg development in females. To verify that variation in ovary size (a measure of the degree of egg development; Trabalon et al. 1992) is correlated with female abdomen size, I analyzed the relationship between abdomen size and ovary mass in wild-caught females. I collected 16 females in Alachua County, Florida between 27 July 2010 and 12 September 2010 and dissected them within 1 day of collection. I placed females in a -20° C freezer for 7 min and photographed and measured female abdomen height (the dorsal-ventral height of the abdomen measured just posterior to the epigynal slit: Vincent & Lailvaux 2006; Rittschof & Ruggles 2010), following Rittschof (2011). I dissected the ovaries out of the abdomen in 10 mM phosphate-buffered saline and preserved them in 95% ethanol. Prior to weighing, I dried ovaries in an oven at 37° C for 30-60 min, depending on size. There was a strong positive relationship ($R^2 = 0.80$, P < 0.0001) between logtransformed ovarian mass (prior to transformation: range: 0.0006-0.2987 g, mean = 0.095 g, SE = 0.03 g) and abdomen height (range: 4.4-11.9 mm, mean = 9.2 mm, SE = 0.55 mm),

supporting my use of adult female abdomen height as a measure of egg development in *N. clavipes*.

Once I had established that abdomen height is an adequate measure of egg development, in a laboratory setting I tested how long-term exposure to low temperature affects changes in female abdomen size, egg development, and the ability to oviposit. I predicted that when kept at low temperatures, female abdomen height would either fail to increase over time, or if it did increase, that this change would not correspond to an increase in ovary size. Finally, I predicted that females kept at low temperatures would fail to reach oviposition. In order to control for laboratory artifacts, I monitored a control group of spiders kept at a warmer temperature (24° C). I collected 16 mature females from the Ordway-Swisher Biological Station in Melrose, Florida (Putnam County, latitude 29°42′32.4″N, longitude 82°2′60.0″W) on 25 Aug 2009. I assigned females to either the cold or warm temperature treatment at random. Because females are typically mated immediately after their maturation molt (Christenson et al. 1985), I assumed mature females were non-virgin. If some females were unmated at the time of collection (which would impact egg development rate: Trabalon et al. 1992), this should not have changed the major results of the experiment because females were assigned to the temperature-controlled rooms (n = 8 females per room) at random. In the cold treatment I kept females at 16° C, which is approximately the minimum daily mean temperature during the month of October (Fig. 2), the last month of the season with an appreciable number of adult females still alive and presumably attempting to produce egg clutches. Females kept in the warmer control room were held at 24° C, which is near the mean temperature for the month of September, when females are still observed to lay eggs in the field (Rittschof & Ruggles 2010). Prior to initiating the experiment, there were no significant differences in abdomen height comparing 16° C and 24° C females (*t*-test, t_{14b} = 1.44, P < 0.25).

From 27 Aug 2009 to 30 Sep 2009, I housed mature female spiders in individual cages (30.5 cm cubes; Bioquip, Rancho Dominguez, CA) in one of the two temperature-controlled rooms (approximately 3.1 m \times 4.6 m \times 2.4 m) maintained at a 12 h light/dark cycle. Humidity could not be controlled within the rooms. Cages were small relative to typical N. clavipes web sizes (average web width is about 99 cm: Vincent & Lailvaux 2006), but given 24 h within these cages in a field setting, all females built prey-capture webs. Within each room I rotated the females' positions every other day in order to control for variation in conditions within the room. I measured the abdomen height for each female from digital photographs each day following Rittschof (2011), and I fed females 1 approximately 2.5 cm long mealworm each day that they had an intact prey-capture web. Previous studies (Rittsehof 2010, 2011) show that caged females kept in outdoor conditions and fed the same diet successfully laid multiple egg clutches at ~25 day intervals. When a female died in the 16° C room, I dissected her abdomen following the protocol for wild females (see above). I did not evaluate female ovary sizes from the 24° C room because, unlike females kept at 16° C, some of these females laid eggs (see below) and thus were at various reproductive stages at the time of death. Overall, 38% of females died within the time frame of the experiment. For

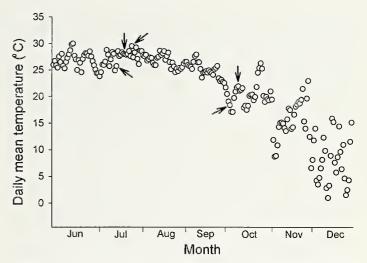


Figure 2.—Daily mean temperatures from the Putnam Hall weather station within the Ordway-Swisher Biological Station. Means were calculated from 96 temperature readings per day taken at 2 m height (the approximate height of webs included in this study). Data were accessed from the Florida Automated Weather Network (University of Florida Institute of Food and Agricultural Sciences Extension). Arrows mark sampling dates (the first of three consecutive days of sampling), 16 Jul 2010, 21 Jul 2010, and 27 Jul 2010 in the early season and 5 Oct 2010 and 10 Oct 2010 in the late season.

those that did not, I kept females in the same treatment conditions until they died in order to calculate a measure of reproductive lifespan, which I defined as the length of time between collection from the wild and death.

There were notable effects of laboratory conditions. Reproductive lifespan across all laboratory animals (n=8 females per room; mean = 27.3 days, SE = 1.8 d) was much shorter than the maximum reproductive lifespan in temperate populations of N. clavipes (as long as 4 months: C. Rittschof personal observation; Brown 1985; Christenson et al. 1985). Female performance, indicated by the percentage of days that females successfully built prey-capture webs and attempted to feed, was similar between the two treatment rooms (16° C: n=8, mean = 52.6%, SE = 10.8%; 24° C: n=8, mean = 49.4%, SE = 12.0%; two-tailed t test, $t_{14}=0.041$, P=0.84), although performance seemed low compared to females kept in outdoor cages or unrestrained females in the wild (C. Rittschof, personal observation).

Despite shorter overall reproductive lifespan in the laboratory, mean reproductive lifespan was significantly longer for females kept at 16° C versus 24° C (mean_{cold} = 31 d, SE = 2.2 days; mean_{warm} = 24 days, SE = 2.2 d; two-tailed t test, $t_{14} = 5.5$, P < 0.034). Prolonged reproductive lifespan in the colder room is in agreement with a fundamental prediction of metabolic theory: metabolism decreases with temperature, resulting in increased lifespan (Brown et al. 2004). Thus this result provides evidence that the low temperature treatment caused metabolic changes in the spiders beyond laboratory artifacts.

Results suggest that decreased temperature may affect female egg development and propensity to oviposit. In the 24° C room, three females successfully laid egg clutches over the experimental period, and one female laid two clutches of

eggs within this time span. In the 16° C room, no females successfully laid eggs over the entire experimental time period (the last female that died was monitored for 38 days). The differences in the occurrence of oviposition were not statistically significant (n = 8 females per room, Fisher's Exact Test, $X^2 = 3.7$, P = 0.1). Even though females failed to lay eggs in the 16° C room, female abdomen height increased over the course of the experiment (n = 8; nested ANOVA with time nested within female identity, $F_{24,175} = 14.2$, P < 0.0001). Maximum female abdomen height in the 16° C room was not significantly different from the 24° C room (16° C room, mean = 9.5 mm, 24° C room, mean = 8.5 mm; $t_{14} = -1.74$, P <0.10), and in contrast to wild-caught females, abdomen height for females kept at 16° C was not positively correlated with ovary mass (n = 7 females because one cold-room female had necrotic ovaries; mean_{AB} = 7.7 mm, range: 4.1–11.0 mm; $mean_{OV} = 0.07$ g, range = 0.002-0.14 g; $F_{1.5} = 1.59$, P <0.26), which suggests that abdomen height is not a valid proxy for ovary development under all conditions. These experimental sample sizes are small, however given the predicted strength of the relationship between abdomen height and ovary mass from field data ($R^2 = 0.8$), and the broad range of abdomen sizes represented in the data set, the regression analysis has a statistical power greater than 0.8 to detect a significant correlation between abdomen size and ovary mass.

Because increased ovary mass is the result of the addition of yolk proteins to eggs (Trabalon et al. 1992), one possible explanation for difference between females housed at 16° C and wild-caught females is that females kept at cold temperatures did not efficiently convert the nutrients they consumed into egg protein as a result of decreased metabolism at low temperature (Li & Jackson 1996; Gillooly et al. 2001; Ladyman et al. 2003). The data suggest that the seasonal decline in temperatures (temperatures drop below 16° C while mature females are still alive, Fig. 2) may have implications for female reproductive success. Egg development could slow or stop due to low temperatures, limiting total reproductive output for late-maturing females (Higgins 2000).

Although the current study suggests that seasonal low temperatures may impede female reproduction in *N. clavipes*, the results are not conclusive. For example, the lack of a correlation between ovary mass and abdomen size in the 16° C laboratory females may be due to laboratory artifacts or small sample sizes. Future studies should assess ovary development at various times throughout the experimental period as opposed to at a single time point at the end. The results presented here suggest that further work is needed to clearly capture the effects of temperature on reproduction in this species.

In summary, the laboratory experiment provides some evidence for temperature-dependent egg development in *N. clavipes*. When females are kept at cold temperatures, abdomen height increases over time, but this increase does not correspond to ovary development, two variables that are highly correlated in wild-caught females. Temperature-dependent egg development may explain the shift in web site preferences at the end of the season when temperature decreases and becomes more variable (Fig. 2). Acceptable web sites in the early season become unsuitable in the late season, perhaps because they have more extreme low tem-

peratures compared to other potential sites (Fig. 1). Web site selection in general may become more critical in the late fall when there is greater variation in temperature within a small geographic area (for example between shaded and sunny sites).

Other studies have shown that spiders have temperaturedependent web site preferences (e.g., Barghusen et al. 1997). In N. clavipes, Higgins and Ezcurra (1996) found evidence in high altitude tropical deserts that females build webs at higher distances from the ground during the late season, presumably as a means to control body temperature. However, here I demonstrate that temperature also varies within a single range of web heights (i.e., ≤ 2 m: Fig. 1), and females may respond to this variation through web site selection. Furthermore, the findings presented here show that females are at times exposed to temperatures prohibitively low for egg development. Future studies in N. clavipes should examine a broader range of temperatures, compare cyclical versus constant temperature regimes, and increase sample sizes in order to further describe how temperature affects egg development. The ability to overcome seasonal temperature constraints is particularly intriguing for a tropical species like Nephila clavipes, whose range has expanded to temperate areas. This species has undergone behavioral and life-history adaptations to the temperate climate; however, individuals may still suffer a late-season reproductive penalty due to insurmountable low temperatures.

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LITERATURE CITED

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SHORT COMMUNICATION

The effect of regurgitated digestive fluid on the spider's own legs in *Philoponella vicina* (Araneae: Uloboridae)

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Abstract. Philoponella vicina O. Pickard-Cambridge 1899 rests on its orb web in a cryptic posture with its legs folded against its body. While feeding, the spider coats the entire prey with digestive fluid and changes its posture, spreading its anterior legs wide. We tested whether this change in leg position may function to protect against damage to its legs from its own digestive fluid. When we touched detached legs I with prey packages wetted with digestive fluid, more setae fell from the legs than when we applied tap water in a similar manner. In addition, intersegmental membranes were damaged by digestive fluid, but not by water. This and other uloborids may thus break their cryptic postures while feeding in order to avoid damage from their own digestive enzymes.

Keywords: Cryptic posture, membrane degradation, setae

The feeding process of uloborids is unusual in several respects. Since these spiders lack cheliceral venom glands, they wrap their prey with large amounts of silk, forming rounded, compact, compressed packages. The spider then repeatedly wets the entire surface of the prey with regurgitated fluid containing digestive enzymes and does not masticate the prey with its chelicerae (Eberhard et al. 2006a). The digestive enzymes must penetrate the prey without the benefit of holes made by the spider while injecting venom or masticating the prey, as occurs in other spiders. The enzymes apparently gain access to the prey's interior by digesting prey membranes, because digested prey showed extensive, membrane-specific damage (Eberhard et al. 2006 a, b). As with other spiders, uloborids have multiple setae around the mouth that presumably function to filter the liquid they ingest (Foelix 2010). Uloborid spiders cover the entire prey package with digestive fluid while feeding, while most spiders wet only the portion close to their chelicerae (Weng et al. 2006).

Philoponella vicina O. Pickard-Cambridge 1899 and other uloborids rest on their webs in various constrained cryptic postures that vary in different genera, but have the common effect of obscuring the outlines of their anterior legs (Opell & Eberhard 1984). In Philoponella, the distal portions of legs I are folded ventrally tight against the body, with their metatarsi and tarsi close to the sternum. This posture is thought to provide protection against visually orienting predators (Opell & Eberhard 1984). This species and other uloborid spiders (i.e., Uloborus trilineatus, U. diversus) break their cryptic postures when feeding, spreading their anterior legs apart (Fig. 1) (Weng et al. 2006; W. Eberhard unpublished results). Weng and coworkers (2006) hypothesized that this spread-leg posture functions to avoid damage to the spider's front legs from its own digestive enzymes.

We used adult female *P. vicina* (length: 5–10 mm), which build approximately horizontal orb webs in sheltered sites in tropical forests and forest edges where they feed on several types of prey (Fincke 1981, Eberhard et al. 2006a), to evaluate the effect of their regurgitated digestive fluid on their own legs. We thus tested the hypothesis that these spiders break their cryptic postures and expose themselves to increased predation in order to avoid possible damage while feeding.

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We collected mature female *Philoponella vicina* in a patch of secondary forest on the campus of the Universidad de Costa Rica in San José, Costa Rica. We induced 15 spiders to build their webs indoors on wire hoops (approximately 20 °C, 80% relative humidity) in order to feed them and to obtain digestive fluids. We sacrificed 13 other mature females by freezing them.

For each experiment, we detached both legs I of a spider and placed them on a glass slide inside a humid chamber (a Petri dish containing cotton soaked in water). These humid conditions slowed the desiccation rate of the regurgitated fluid; otherwise, when it was extracted and exposed to air, it dried in a few seconds. One of the legs (the "experimental" leg) received regurgitated digestive fluid, while the other (the "control" leg) received tap water. To obtain digestive fluid, we fed prey (wild Drosophila flies or Tetragonisca stingless bees) to spiders in their webs. We pulled the prey from the spider's grasp with a pair of forceps after the spider had wrapped the prey and wet it with digestive fluid (the prey package changed from opaque white to translucent and shiny) (Eberhard et al. 2006b). We removed the prey while the spider was regurgitating and rotating the package to wet it. This behavior precedes ingestion, which begins when the spider stops rotating its wetted prey. We applied digestive fluid (or water) from five different prey packages to each leg. Each package was touched to the leg at five different spots, sufficient to wet the entire surface of the leg as the liquid dispersed. The touches were gentle, and the entire surface of the leg became wet, but it was not possible to be sure that equal amounts of water and digestive liquid were applied. No region of the leg was touched preferentially. Each application lasted for about 30 s, until the liquid evaporated.

To control for the possibility that setae were lost from the leg due to the mechanical stress produced when we touched the legs with prey packages, we used packages of prey taken from spiders before they had regurgitated and which we wet with water. We alternated the legs receiving treatments (experimental, control, experimental, etc.). As it was necessary to wait for spiders to wrap and begin to feed on new prey, the lapse of time between repeats of the treatments was not uniform, but on the order of 10 min.

We then observed the two legs under a dissection microscope at 100X. Counts of fallen setae were made in the white ring on tibia I, where individual setae and their sockets were easy to distinguish. All intersegmental membranes were checked for damage, such as holes or complete separation of leg segments (Fig. 2b). We calculated the area of this white ring for one side of the leg, using a calibrated ocular

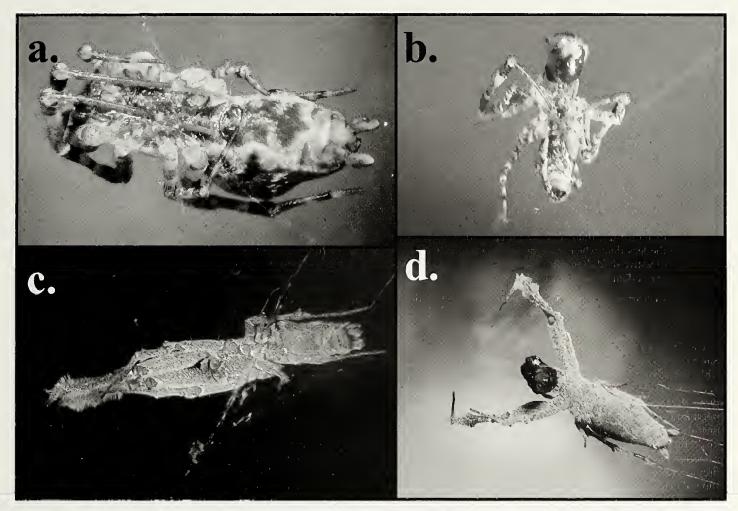


Figure 1.—Ventral views of *Philoponella vicina* on its web while a) resting and b) feeding. Ventral views of *Uloborus trilineatus* while c) resting and d) feeding. Note that the anterior legs are spread while feeding in both species.

micrometer with a resolution of $0.5~\mu m$. To calculate the area, we multiplied the width of the leg times the length of the white ring. We counted the number of intact tibial setae in that area and the number of pores from which setae had fallen (Fig. 2a).

We calculated the proportion of setae that were lost per area in each treatment. We used proportions to avoid possible effects of individual variation. We compared the proportions with a Wilcoxon



Figure 2.—a) Pores left by fallen setae (arrows) on the tibia of leg I of *Philoponella vicina* resulting from the application of its own digestive fluid (detail of pores at upper left); b) Damage (arrow) to the membrane at the femur-patella articulation of leg I that had been treated with regurgitated digestive fluid.

test. All means are reported with one standard deviation. Voucher specimens are housed in the Museo de Zoología (USJ) at the Universidad de Costa Rica.

The mean white area of leg I exposed to the digestive fluid treatment was $160 \pm 42.3 \, \mu m^2$ (n=18), and the mean setae density was 0.31 ± 0.06 setae / μm^2 . More setae were missing following the digestive fluid treatment than following the tap water treatment (Z=2.69; P=0.007; Fig. 3) (the treatment variances were equal - Levene's F=2.04; gl=12; P=0.23; Fig. 3). Six of 13 legs exposed to the regurgitated fluid had damage in at least one joint membrane (Fig. 2b), but no legs exposed to tap water showed any deterioration. The femur-patella joint was the most frequently damaged, with six legs affected; four legs were damaged at the tibia-metatarsus joint, and four legs at the patella-tibia joint.

Thus the legs of *P. vicina* are susceptible to injury from the digestive enzymes that the spider applies to its prey. Some setae also fell out with the tap water treatment, probably due to the mechanical effect of our applying the wetted prey package to the legs. The sharpest negative effect of the regurgitated digestive fluid was the damage to the joint membranes, which only occurred when regurgitated fluid was applied. This effect would probably be severely damaging to a living spider.

It is likely that the change in resting posture during feeding functions to prevent contact of the spider's legs with the regurgitated liquid that covers the prey package. This could be especially important, because these spiders spend up to several hours feeding on a given prey (Eberhard et al. 2006a) and because their digestive

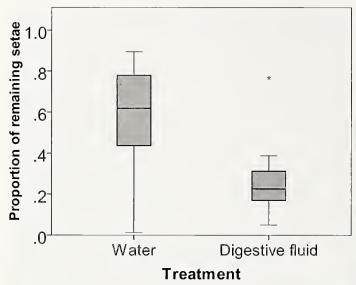


Figure 3.—Proportion (median \pm percentiles and range) of sctae that were missing from tibia 1 of *Philoponella vicina* after applying its own digestive fluid (left) or tap water (right) (Z = 2.69, P = 0.007).

fluid has a low surface tension, which aids it in wetting and digesting the prey (Weng et al. 2006).

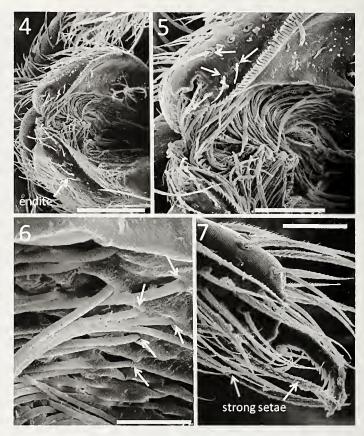
These considerations bring into focus additional feeding problems for uoborids that we will only mention briefly. The setae near the spider's mouth differ from those on other parts of the body in not having conspicuous sockets at their bases (Figs. 4-6). Perhaps this design serves to avoid membrane damage from digestive fluids. The fact that these setae arc intact (Figs. 4–6), despite having been repeatedly exposed to digestive fluid during the spider's lifetime, makes it clear that they are not damaged by digestive fluids. In contrast, the mouth setae of an araneid, Argiope argentata, that envenomates and then masticates its prey and thus may have less need to digest prey membranes, differ from those of P. vicina in having clear sockets. In addition, the palps of P. vicina, which manipulate the wet prey during regurgitation, are provided with long, robust setac at their tips (Fig. 7), perhaps to help prevent digestive fluids from contacting the rest of the palp when the spider rotates the prey while feeding. Further observations and comparisons with other species will be needed to test these hypotheses.

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Figures 4–7.—SEM images of setae near the mouth and on the palp of *Philoponella vicina*. Arrows in Figure 5 mark "typical" setal bases with clear sockets on the distal edges of the endites. Arrows in Figure 6 mark the less distinct sockets at the bases of setae on the dorsal surfaces of the endites near the mouth. Arrows in Figure 7 mark the two robust setae near the tarsal claw, which were observed to repeatedly contact the prey package as it was rotated during feeding. Scale lines are, respectively, 150, 60, 15, and 43 μ long.

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